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Waterloo Centre for Process Development

BIOTECHNOLOGICAL METHODS FOR UPGRADING OF BITUMEN, HEAVY OIL AND RESIDUE

FINAL REPORT FOR PHASE II

by

George Ching, Suzanne Rioux, Jeno M. Scharer and M. Moo-Young

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TABLE OF CONTENTS

		Page
ABSTRACT		1
INTRODUCTION		3
PRELIMINARY STUDIES		5
FERMENTOR STUDIES		10
DISCUSSION		36
CONCLUSIONS AND RECOMMENDATIONS		44
REFERENCES		47
APPENDIX I	Materials and Methods	48
APPENDIX II	Analytical Techniques	50
APPENDIX III	Cultures and Media	53
APPENDIX IV	Raw Data	59

ABSTRACT

Phase II of the research contract concentrated on establishing the most suitable organisms and growth conditions for microbially upgrading Lloydminster crude. A series of comparative experiments were performed, using a variety of bacterial species and growth conditions identified in Phase I for both Lloydminster crude and a synthetic analogue. These early experiments indicated a superiority of one organism, Arthrobacter paraffineus, in viscosity and interfacial surface tension reduction and for sulphur removal. Optimum growth conditions and medium compositions were determined.

In a series of studies aimed at optimizing the performance of Arthrobacter paraffineus in a continuously stirred tank reactor, various combinations of media, aeration, and growth conditions were investigated using Lloydminster crude as a substrate. It was found that 12% of the organic sulphur could be removed during a six-day incubation period under aerobic conditions. Sulphur removal was dependent on growth conditions. Comparative studies under anaerobic conditions were performed with Desulfovibrio desulfuricans. Anoxic conditions were maintained by sparging the continuous stirred reactor with nitrogen. The sulphur removal ranged from 10.7% in synthetic media to 0.17% sulphur from crude oil. The low

sulphur removal from crude oil was attributed to growth-inhibitory compounds in the crude. These preliminary results indicate that limited sulphur removal from crude oil is possible using monocultures of Arthrobacter paraffineus or Desulfovibrio desulfuricans. A concurrent beneficial effect is a reduction of viscosity (by biosurfactants) and surface tension obtainable under these conditions. Further studies are recommended using immobilized biomass, mixed cultures, and other bacterial species.

INTRODUCTION

Canada has large deposits of heavy oils and bitumen. Typically, these oils contain 3 to 6% sulphur. Sulphur is present in both organic and inorganic compounds, organic sulphur compounds being the most abundant. Over 200 different organic compounds, specifically thiols, thiophenes, substituted benzo- and dibenzothiophenes have been identified in various heavy oils and bitumen. In many high sulphur crude oils, dibenzothiophenes (DBT) comprise the major organic fraction. Many polycyclic thio compounds (dibenzothiophenes, for example) are particularly stable and tend to enrich in the residual oil during thermal cracking (Finnerty, 1982). These objectionable quantities of sulphur must be removed by some desulphurization technology. The physico-chemical processes of desulphurization require high temperatures, high pressures and catalysts. Conventional hydrocracking for sulphur removal is rather expensive; it is estimated that it may add $10-$16 per bbl to the cost of refining (Hartdegen et al., 1984).

The application of microbial biotechnology to upgrade high-sulphur crude oils has been discussed in the recent scientific literature. The review of this literature on the biotechnological methods for upgrading heavy oil, bitumen, and residues has been the subject of Phase I of the present contract research (Bhadra et al., 1986). Both aerobic and anaerobic microbial processes for desulphurization and viscosity reduction have been reviewed. It was noted that most previous work has been performed with model substrates, particularly DBT in synthetic medium. Only a few studies involved actual crude oil upgrading. These studies have

not resulted in commercial microbial process development, since the engineering aspects of the process (optimal conditions, reactor design, scale-up) have not been addressed in sufficient detail. The previous research, however, was used to identify the most promising bacterial species and growth conditions for Phase II of this research.

The primary objectives of Phase II of the research contract was to perform comparative studies and engineering evaluation of microbial desulphurization and viscosity reduction using the most promising organisms as identified in Phase I of the study. These studies were performed in media containing Lloydminster crude oil. On the basis of the preliminary results, two types of fermentor design, namely pneumatically mixed and stirred tank reactors, were tested to assess the equipment design aspects and operation conditions.

PRELIMINARY STUDIES

The bacterial species employed in this study included the following:

Arthrobacter paraffineus

Desulphoribrio desulphuricans

Pseudomonas aeruginosa

Pseudomonas alcaligenes

Pseudomonas putida

Rhodococcus erythropolis

Sulfolobus acidocaldarus

The selection of the microorganisms was based, in part, on the recommendations of the Phase I of this study (Bhadra et al., 1986). The bacteria Pseudomonas alcaligenes and Sulfolobus acidocaldarus had been identified for their ability to oxidize organic sulphur compounds, while Arthrobacter parafinneus, Rhodococcus erythropolis and Pseudomonas aeruginosa had been studied previously for surfactant production. The microorganism, Pseudomonas putida was added, because its ability to metabolize a wide range of alkyl and aryl hydrocarbons is well known. Desulfovibrio desulfuricans was issued in Germany (Eckart et al., 1986). The inoculum for the preliminary experiments was prepared by pre-culturing the various species in bacteriological culture tubes. The preliminary studies were carried out in 250 ml shake flasks (Erlenmeyer flasks) containing 85 ml aqueous medium and 15 ml oil. Medium formulations were derived from published data and recommendations of the American Type

Culture Collection Manual . The media used in this study are summarized in Appendix III.

After a prescribed incubation period the contents were subjected to tests including dissolved sulphates in the aqueous phase, surface and interfacial tensions of the liquids, viscosity of the oil, cell growth, critical micelle concentration of biosurfactants, and pentane/toluene insolubles concentration. A detailed description of the materials and methods involved is given in Appendix I.

Biological sulphur removal from crude oil is accomplished by the conversion of insoluble thiophenes to water soluble sulphur compounds. In this study the rise in concentration of dissolved sulphates was used to determine sulphur removal from oil. Results of comparative desulphurization experiments under aerobic conditions are summarized in Table 1, and illustrated in Figure 1. Of the aerobic species, only Arthrobacter paraffineus and Pseudomonas putida had apparent oxidative activity.

Under the conditions of this experiment Arthrobacter paraffineus was clearly more capable of removing sulphur from crude oil than were the other organisms studied. The bacter, Pseudomonas alcaligenes and Pseudomonas aeruginosa were unable to oxidize the sulphur moiety. These organisms may have produced some soluble intermediates that were not detectable by the sulphate analysis. However, partially degraded organic sulphur compounds may cause polutional problems in the aqueous phase. We were not able to

produce an inoculum of the thermophile, Sulfolobus acidocaldarus in a medium containing oil.

The effects of the various species upon the rheological, or fluid, properties of crude oil were then studied. For our purposes, the interfacial tension between the oil and aqueous phases is of most practical interest. It is believed that a reduction of interfacial tension results in better contact between the bacterial surface and the oil. The results tabulated in Table 2 and illustrated in Figure 2 shows Arthrobacter paraffineus to be the most potent organism in this respect as well. On the basis of the experiments discussed above, Arthrobacter paraffineus was selected for closer examination.

The ability of Arthrobacter paraffineus to remove sulphur when grown on a minimal salts medium, with and without added sulphate, is summarized in Table 3. Figure 3, drawn from these results, shows a strong increase in percentage of sulphur removed with the addition of sulphate to the growth medium. There is a concurrent drop in medium pH. Trace levels of yeast extract appeared to positively influence the results in only one case, when used with MSM + SO_4 medium at 25° for 12 days. As shown in Figure 4, there is no overall influence on sulphur removal by yeast extract.

Four replicate samples of oil emulsions from shake flask cultures of Arthrobacter paraffineus were submitted for sulphur analysis to the Energy Technology Branch, CANMET. The cultures were incubated for ten days in medium containing 15% crude oil (no sulphate added). At CANMET, the samples were warmed to reduce the viscosity and centrifuged for 1½ hours to remove the water. The samples were then analyzed for sulphur content. The

results are summarized in Table 15. These results suggest that very little sulphur, if any, was removed from the crude oil phase. The apparent decline in the sulphur content may not be significant, since it may be due to the excess water in the oil emulsion which was due to the emulsifying agents produced by the bacteria.

The influence of sulphate in the medium upon the rheological effects of Arthrobacter paraffineus was also studied. There was a large variance in interfacial tension values, summarized in Table 4, but a positive correlation between sulphate addition and interfacial tension is apparent, as graphed in Figure 5. The surface tensions of oil and medium do not appear to be affected by sulphate addition.

In summary, Arthrobacter paraffineus grew at the temperature range of 25°C to 30°C at pH=7.0. No added carbon source was found to be necessary when grown on Lloydminster crude. A requirement was found for low levels of supplementary vitamins or other growth factors. Approximately 0.05g of yeast extract per litre of medium was sufficient. Sulphate ion in the aqueous medium appeared to stimulate sulphur removal from crude oil.

TABLE 15

SULPHUR CONTENT OF CRUDE OIL SAMPLES TREATED WITH BACTERIA

SAMPLE	SULPHUR CONTENT (%)
UNTREATED OIL	3.69
TREATED OIL SAMPLES	
SAMPLE #1	3.25
SAMPLE #2	3.56
SAMPLE #3	3.31
SAMPLE #4	3.61

FERMENTOR STUDIES

The first three CSTR runs compared the performance of Arthrobacter paraffineus in mineral salts medium with and without added sulphate and with and without aeration. The results of these experiments are tabulated in Tables 5, 6 and 7.

Figure 6 compares the effect of aeration and of sulphate in the medium upon oil viscosity. An initial increase in viscosity was observed, followed by a drop at about 48 h for both treatments. With sulphate in the medium, oil viscosity returned to near the initial value, while it remained high without sulphate.

As illustrated in Figure 7, oil surface tension rose with an aerated and non-sulphate medium, again with a drop near 48 h. No significant effect by sulphate without aeration, or by aeration alone, was observed.

Interfacial tension showed a similar pattern. A rise was seen, interrupted at 48 h, with the aerated, non-sulphate medium. The non-aerated media, with and without sulphate, showed little effect, as seen in Figure 8.

Surface tension of the media was not affected significantly by any of the treatments (Figure 9).

Bacterial growth, as measured by dry weight, reached similar peak values of about 0.5 grams per litre for all three runs, (Figure 10) but at different times. Without aeration or sulphate, growth peaked at about 24 hours. With both, the peak came at 48 hours. With sulphate but not aeration, highest values were recorded at 72 hours.

As shown in Figure 11, there was little change in medium pH in the non-aerated reuns, with or without sulphate. With air and sulphate, there was a steady drop from pH 7 to about pH 4 over a period of 72 hours.

The amount of pentane insolubles present remained similar for each medium throughout the experiments. There was no significant change for 72 hours, followed by a sharp rise (Figure 12).

The results of experiments with Arthrobacter paraffineus in the airlift fermentor are presented in Tables 8 and 9. These experiments were terminated early when the fermentor became choked with a thick mass of oil. While they were running, the removal of sulphur was minimal in both cases. Interfacial tension was not greatly affected, nor was oil surface tension. Oil viscosity, as mentioned, became so great as to force termination of both runs.

Desulfovibrio desulfuricans was inoculated into a CSTR to examine its performance. Nitrogen again was used to agitate since the organism is an anaerobe. In case of Desulfovibrio desulfuricans, of course, the potential sulphur removal was evaluated in terms of sulphide levels in the aqueous

phase. This experiment was also terminated early by a thick agglomeration of oil. As shown in Table 10, there was no significant effect upon any of the parameters being followed in this study.

A pair of CSTR runs were made to study the effect of the oil to medium ratio upon the action of Arthrobacter paraffineus. Table 11 summarizes the results over 10 days with 10% crude oil in an MSM + YE medium. Oil/medium interfacial tension was slightly lower with the smaller amount of crude in the reactor, as shown in Figure 13. Oil viscosity did not vary much with 10% crude in the CSTR, but gradually rose with 20% in the mixture, as shown in Figure 14. Oil surface tension remained relatively constant in both cases (Figure 15). Sulphur removal was minimal in both runs, although somewhat higher with the higher proportion of crude, as shown in Figure 16.

The most recent CSTR studies have employed a synthetic analogue of crude oil as the substrate. A saturated solution of dibenzothiophene in paraffin oil has been employed, with 20% oil. Results of this run are presented in Table 12. Figure 17 shows the sulphur removal over the course of the exeriment, as reflected by the rise in sulphate concentration. The sulphur content of the oil phase was not known. Consequently, the percent sulphur removal could not be determined exactly. A peak in sulphate level is reached at about 48 hours, as is the case with oil surface tension (Figure 18), medium surface tension, (Figure 19), and interfacial tension measurements (Figure 20). Oil viscosity was lowest at 48 hours (Figure 21), and cell growth, as indicated by medium optical density, was greatest (Figure 22).

FIGURE 1 : SULPHUR REMOVAL FROM CRUDE

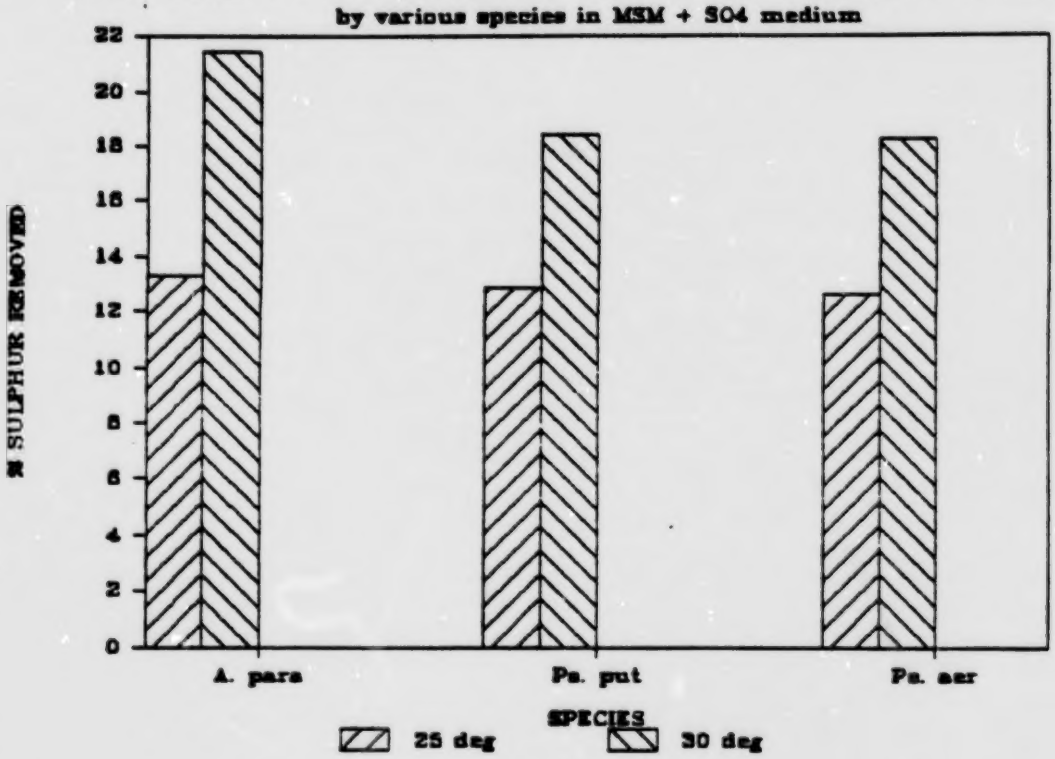


TABLE 1:
SULPHUR REMOVAL FROM CRUDE OIL BY VARIOUS SPECIES

crude oil 15% in shake flasks, 12 days, MSM + SO4 MEDIUM

SPECIES	MEDIUM	TEMP.	INIT pH	FINAL pH	SULPHUR REMOVAL %
Arthrobacter paraffineus	MSM	25C	7.00	4.44	0.29
	MSM + SO4	25C	7.00	4.33	13.32
	MSM	30C	7.00	5.39	1.36
	MSM + SO4	30C	7.00	4.89	21.43
Pseudomonas putida	MSM	25	7.00	6.90	0.29
	MSM + SO4	25	7.00	6.69	12.84
	MSM	30	7.00	6.67	0.28
	MSM + SO4	30	7.00	6.63	18.46
P. aeruginosa	MSM	30	7.00	6.96	NA
	MSM + SO4	30	7.00	6.92	12.64
	MSM	37	7.00	7.02	0.33
	MSM + SO4	37	7.00	6.89	18.3
P. alcaligenes	CITRATE	30	7.00	9.18	1.16
	L & C	30	7.00	9.27	0.32

FIGURE 2: RHEOLOGICAL PROPERTIES

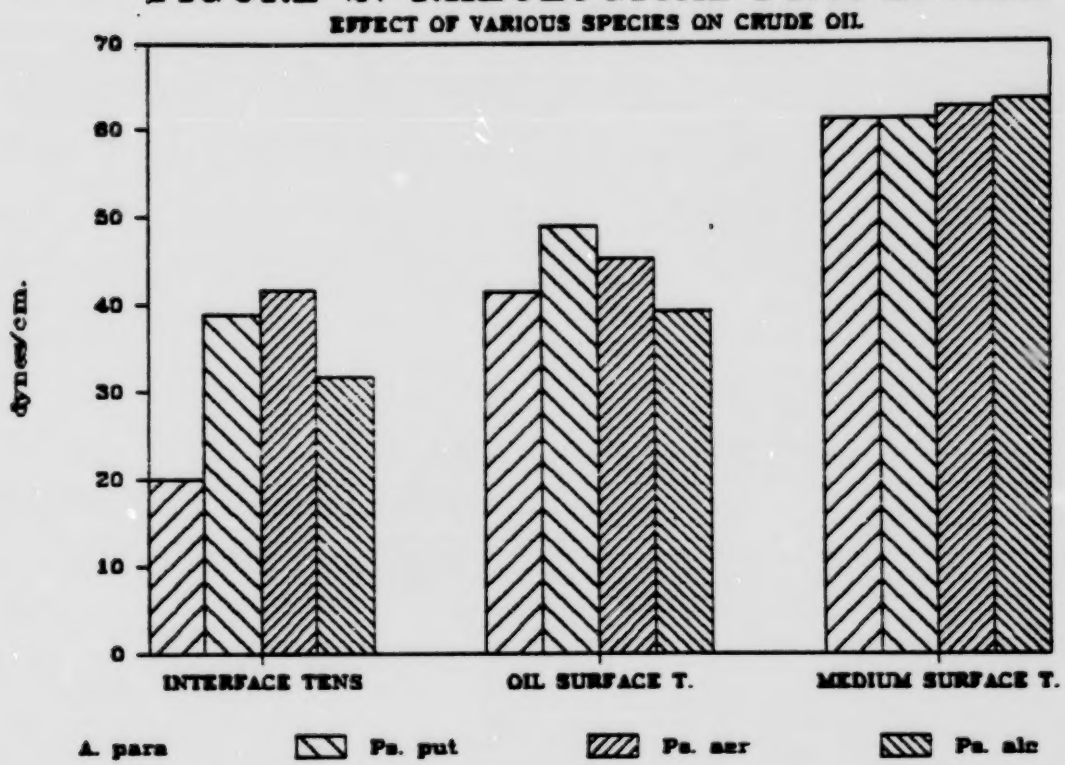


TABLE 2: RHEOLOGICAL PROPERTIES OF CRUDE OIL
EFFECT OF VARIOUS SPECIES

species	INTERFACE TENS	OIL SURFACE T.	MEDIUM SURFACE T.
		dynes / cm.	
CONTROL	32.1	38.1	71.0
A. paraffineus	20.1	41.2	61.1
Ps. putida	38.7	48.9	61.2
Ps. aeruginosa	41.4	45.2	62.6
Ps. alcaligines	31.9	39.1	63.4

Grown 6 days, 15% crude, MSM + SO4 medium (Ps. alc: CITRATE)

TABLE 3: EFFECT OF SULPHATE AND YEAST EXTRACT IN MEDIUMIUM

DURATION days	MEDIUM	YEAST EXTRACT g/l	FINAL pH	SULPHUR REMOVAL %	TEMP. C
6	MSM	0.10	4.97	1.06	25
6	MSM	0.50	5.37	1.43	25
6	MSM + SO4	0.50	5.35	20.28	25
12	MSM	0.05	5.20	0.69	25
12	MSM	0.10	4.73	0.16	25
12	MSM	0.50	5.03	0.26	25
12	MSM + SO4	0.00	4.64	0.38	25
12	MSM + SO4	0.10	4.47	17.71	25
12	MSM + SO4	1.00	5.32	25.01	25
12	MSM*	0.05	7.00	0.36	25
12	MSM*	0.01	7.00	0.03	25
12	MSM	0.50	5.33	0.34	30
12	MSM + SO4	0.00	4.51	22.12	30
12	MSM + SO4	0.10	4.72	20.28	30
12	MSM + SO4	0.50	5.29	22.00	30
12	MSM + SO4	1.00	5.60	18.84	30

* = Control, no inoculum
Crude 15 % in all samples, initial pH 7.00

Fig. 3: EFFECT OF SULPHATE IN MSM

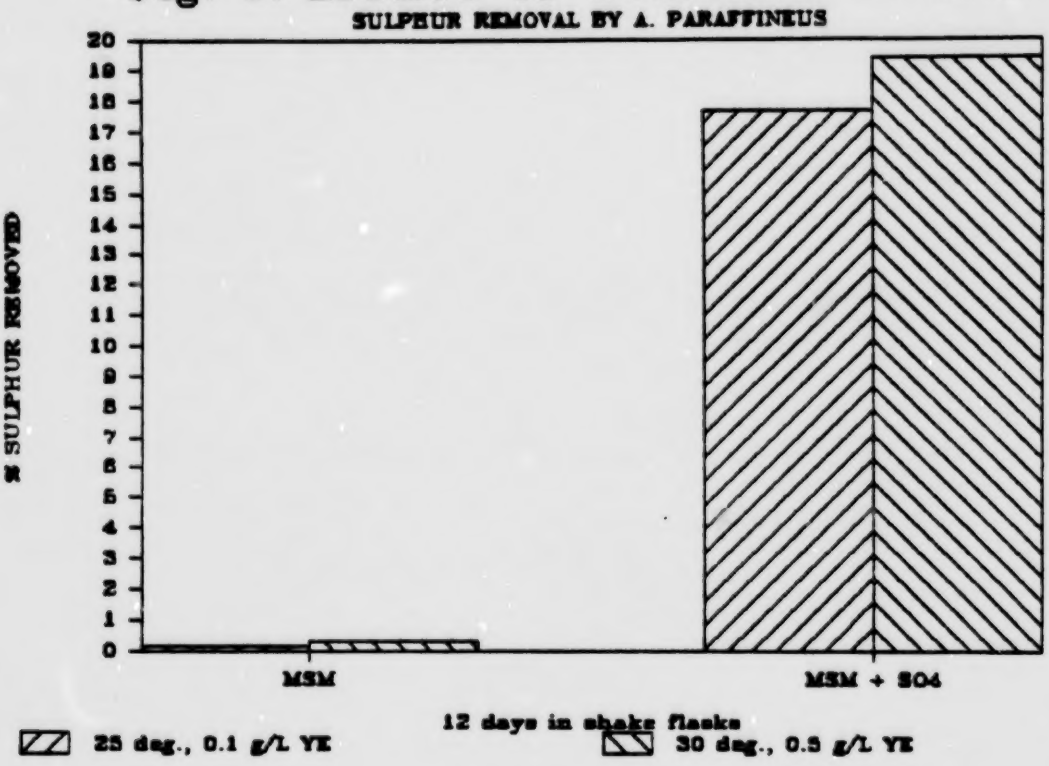


Fig. 4: EFFECT OF Y. E. IN MSM+SO4 MED

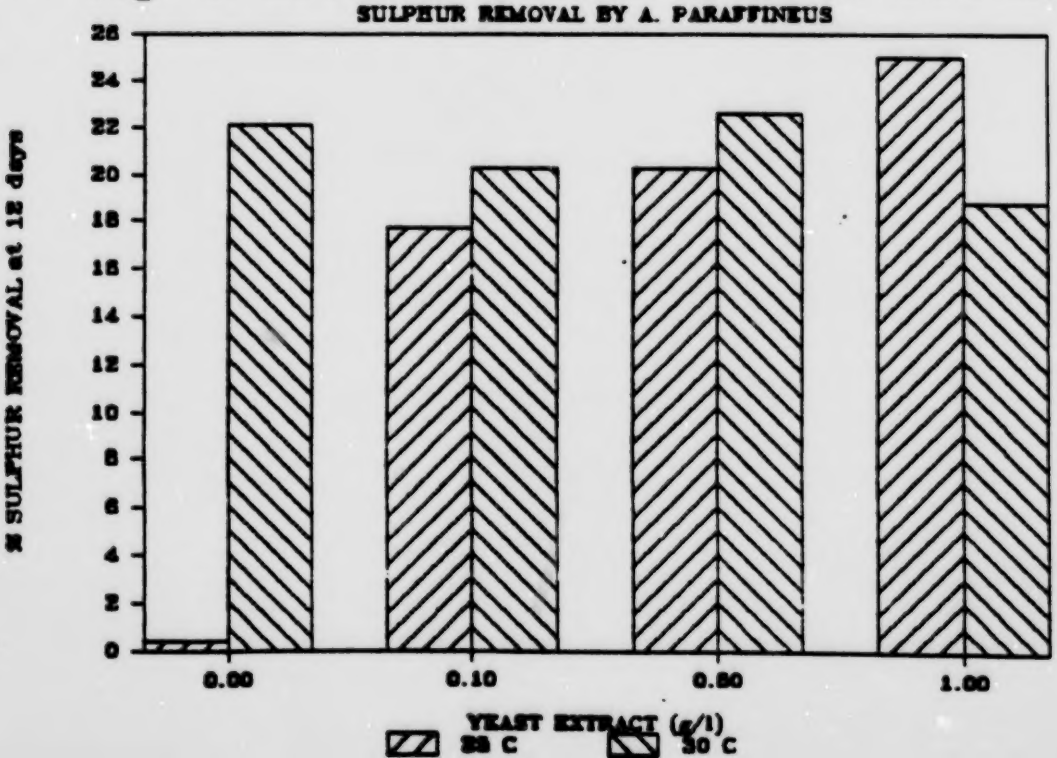


Fig. 5: INFLUENCE OF MEDIUM–RHEOLOGICAl

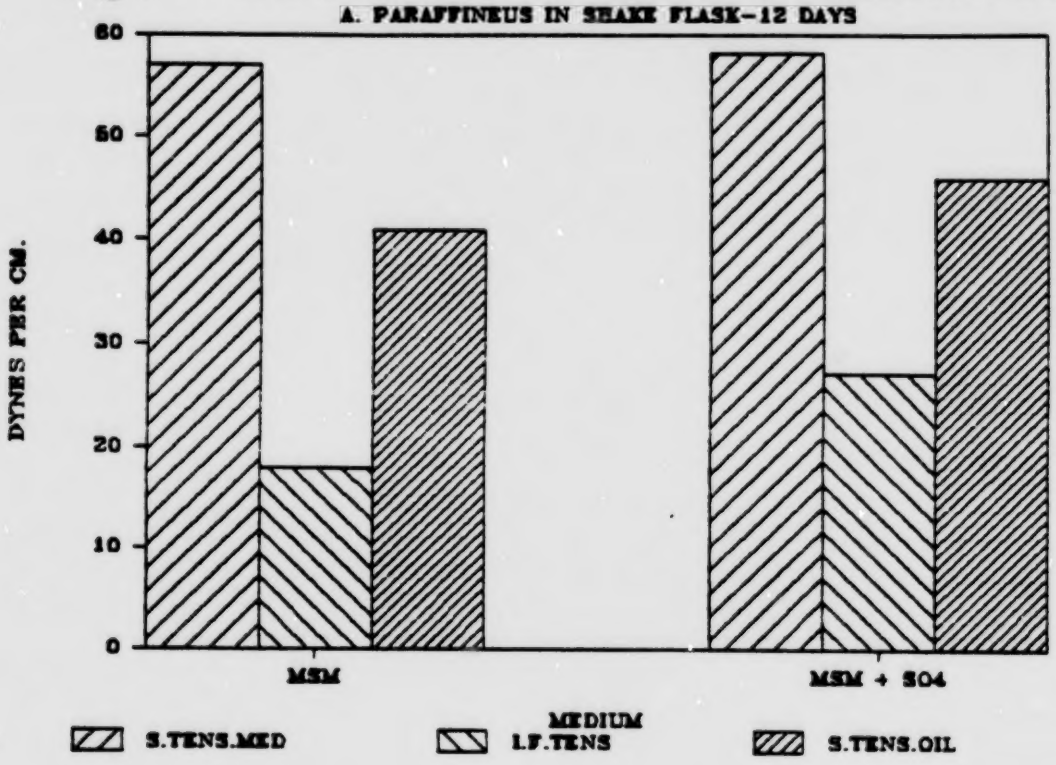


TABLE 4:
RHEOLOGICAL PERFORMANCE EFFECTS OF MEDIUM ON A. PARAFFINEUS

SHAKE FLASKS, 30 DEG., 15% INOCULUM, INIT. pH 7.0

DURATION days	MEDIUM	SURFACE TENSION MEDIUM dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION OIL dynes/cm
12	MSM	57.0	17.9	41.1
12	MSM + SO4	58.1	27.1	46.1

TABLE 5:

SULPHATE AND AIR IN MEDIUM
A. paraffineus with crude oil in CSTR

PARAMETERS	INITIAL	24 HOURS	48 HOURS	72 HOURS	96 HOURS
pH	7.00	6.48	5.29	3.86	3.86
TEMPERATURE C	30.0	30.5	30.0	30.5	29.0
DRY WEIGHT g/l	0.00	0.30	0.50	0.10	0.20
SULPHUR REMOVAL %					
VISCOSITY CStokes	457.59	NA	NA	NA	NA
SURFACE TENSION MEDIUM	70.50	64.40	61.80	65.30	70.30
INTERFACIAL TENSION	32.10	68.40	44.00	59.70	86.50
SURFACE TENSION OIL	37.90	68.50	52.50	76.50	69.00
PENTANE INSOLUBLES	16.24	6.27	6.79	9.53	50.67

MEDIUM USED: MSM + SO4/2 + YE/2 (4 Litres)
OIL SOURCE: LLOYDMINSTER CRUDE (1 Litre)
AERATION RATE: 0.6 v/v
AGITATION RATE: 295 rpm
INOCULUM: 5%

TABLE 6:

SULPHATE, NO AIR IN MEDIUM
A. paraffineus with crude oil in CSTR

PARAMETERS	INITIAL	24 HOURS	48 HOURS	72 HOURS	96 HOURS
pH	7.00	6.43	6.27	6.23	6.29
TEMPERATURE C	30.0	30.3	31.0	28.5	28.0
DRY WEIGHT g/l	0.00	0.40	0.40	0.50	0.40
SULPHUR REMOVAL %					
VISCOSITY CStokes	457.59	612.44	478.55	507.83	467.29
SURFACE TENSION MEDIUM	70.50	55.70	60.90	61.40	61.00
INTERFACIAL TENSION	32.10	25.90	30.30	32.70	29.50
SURFACE TENSION OIL	37.90	34.10	37.00	29.70	38.50
PENTANE INSOLUBLES	16.24	15.87	17.90	11.04	62.30

MEDIUM USED: MSM + SO4/2 + YE/2 (4 Litres)
OIL SOURCE: LLOYDMINSTER CRUDE (1 Litre)
AERATION RATE: 0.0 v/v (none)
AGITATION RATE: 295 rpm
INOCULUM: 5%

TABLE 7:

NO SULPHATE, NO AIR IN MEDIUM
A. paraffineus with crude oil in CSTR

PARAMETERS	INITIAL	24 HOURS	48 HOURS	72 HOURS	96 HOURS
pH	7.00	6.65	6.39	6.30	6.45
TEMPERATURE C	30.0	28.0	30.5	30.0	27.5
DRY WEIGHT g/l	0.00	0.50	0.20	0.10	0.10
SULPHUR REMOVAL %	0.00	0.67	0.18	0.13	0.14
VISCOSITY CStokes	457.59	647.45	578.89	665.47	NA
SURFACE TENSION MEDIUM	70.50	58.90	63.90	61.90	67.80
INTERFACIAL TENSION	32.10	26.60	34.80	34.20	NA
SURFACE TENSION OIL	37.90	37.80	37.20	31.70	68.20
PENTANE INSOLUBLES	18.53	21.40	14.81	11.51	NA

MEDIUM USED: MSM + YE/2 (4 Litres)
OIL SOURCE: LLOYDMINSTER CRUDE (1 Litre)
AERATION RATE: 0.0 v/v (none)
AGITATION RATE: 295 rpm
INOCULUM: 5%

Fig. 6 :OIL VISCOSITY

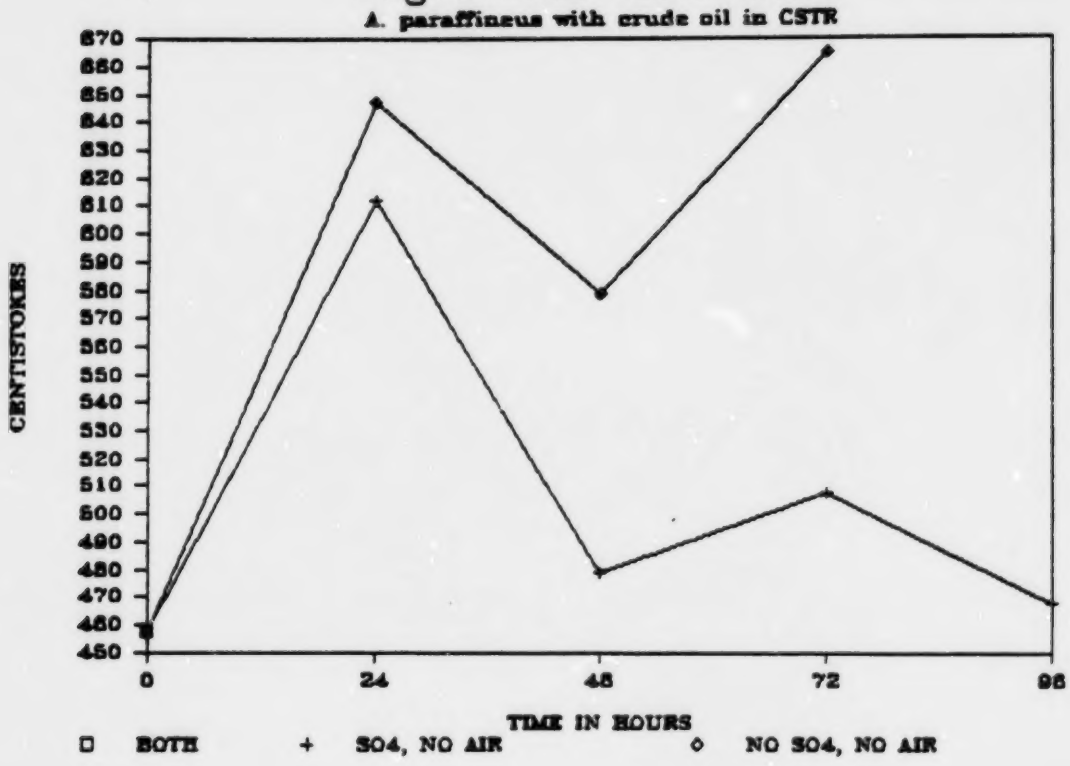


Fig. 7: OIL SURFACE TENSION

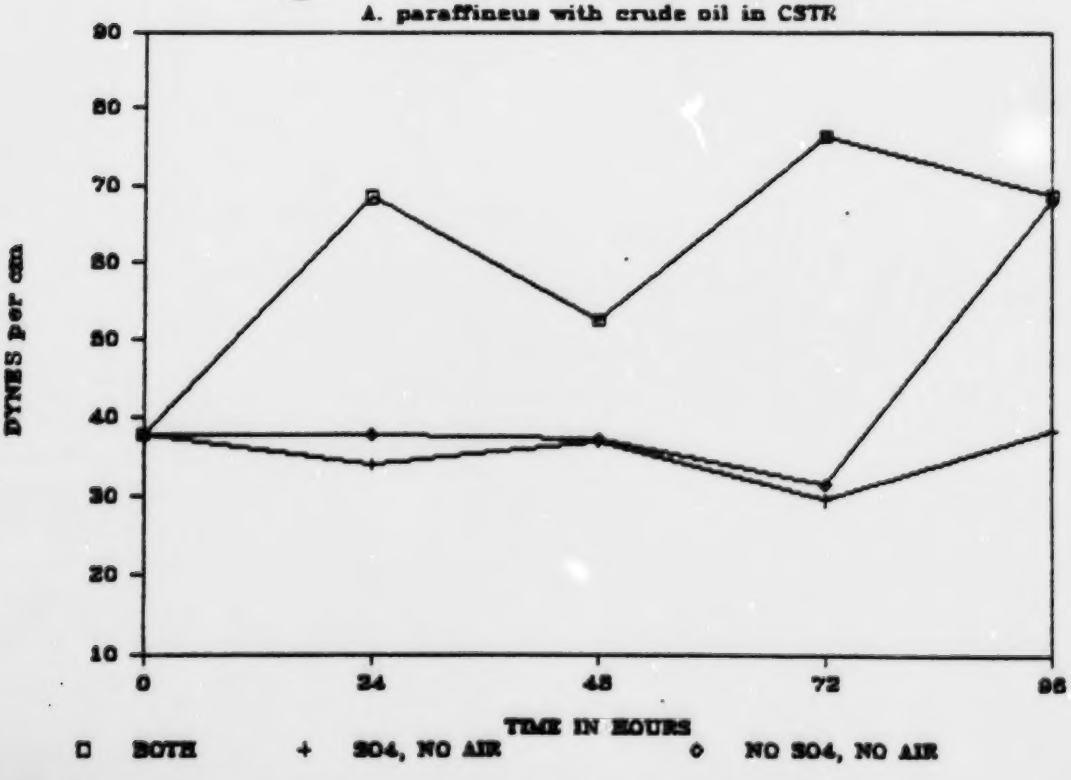


Fig. 8 :OIL MEDIUM INTERFACIAL TENSION

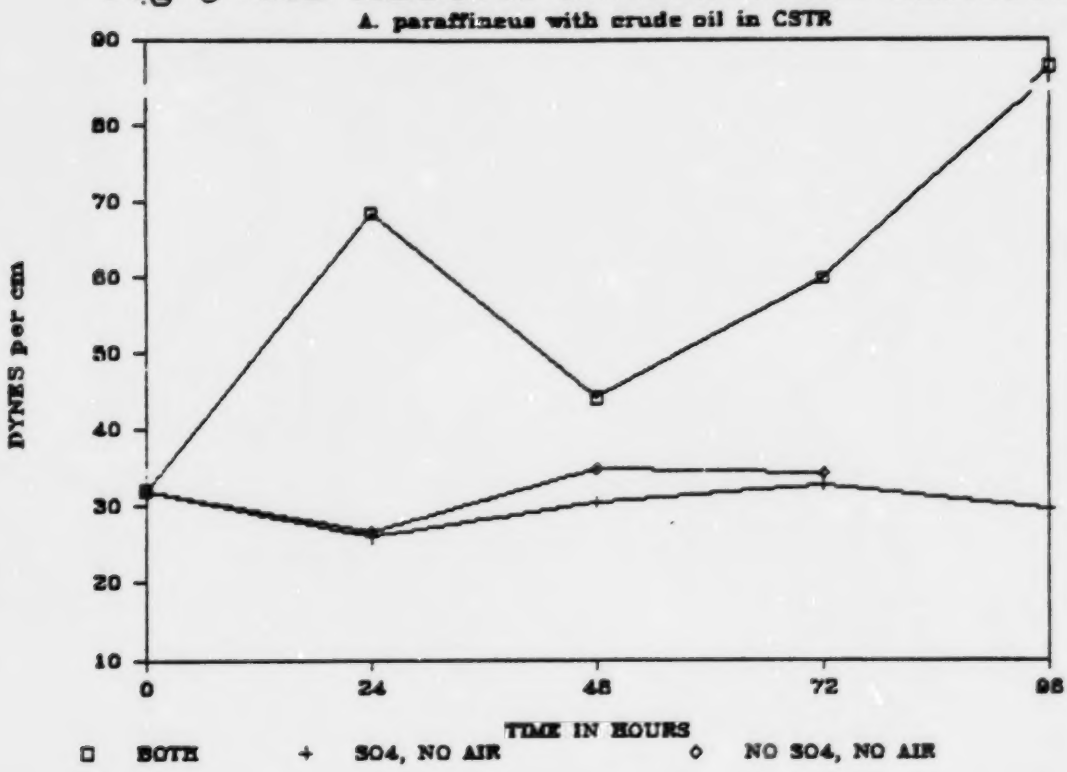


Fig. 9 : MEDIUM SURFACE TENSION

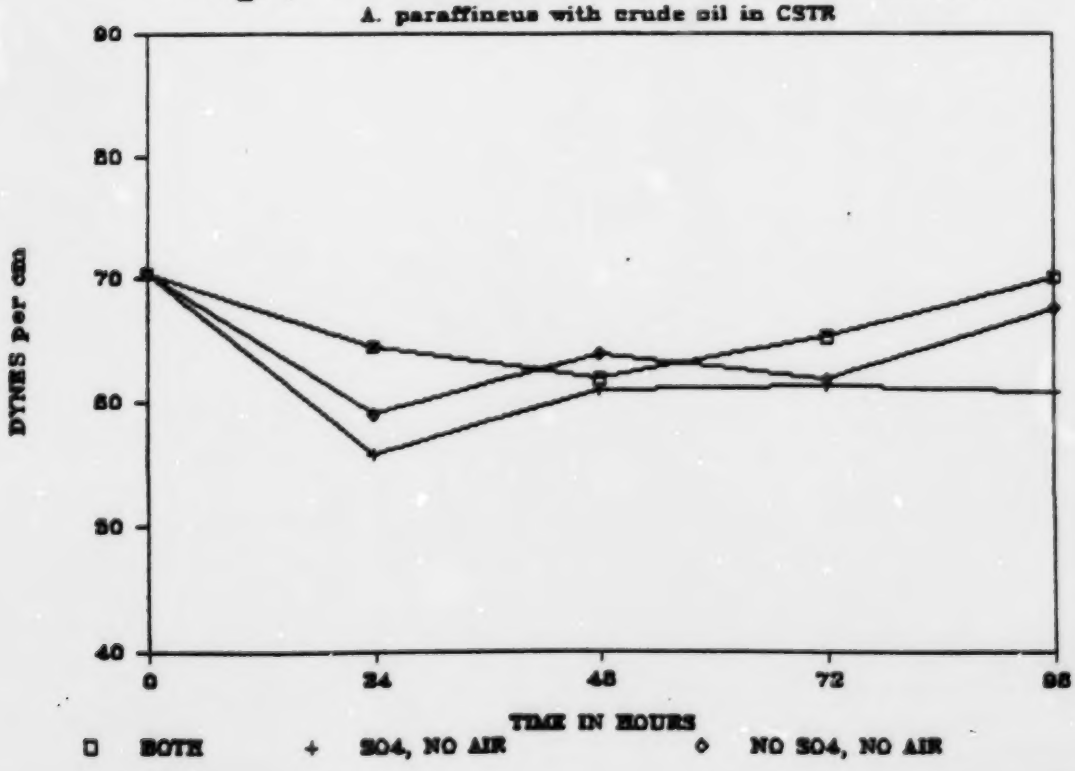


Fig. 10: CELL GROWTH AS DRY WEIGHT

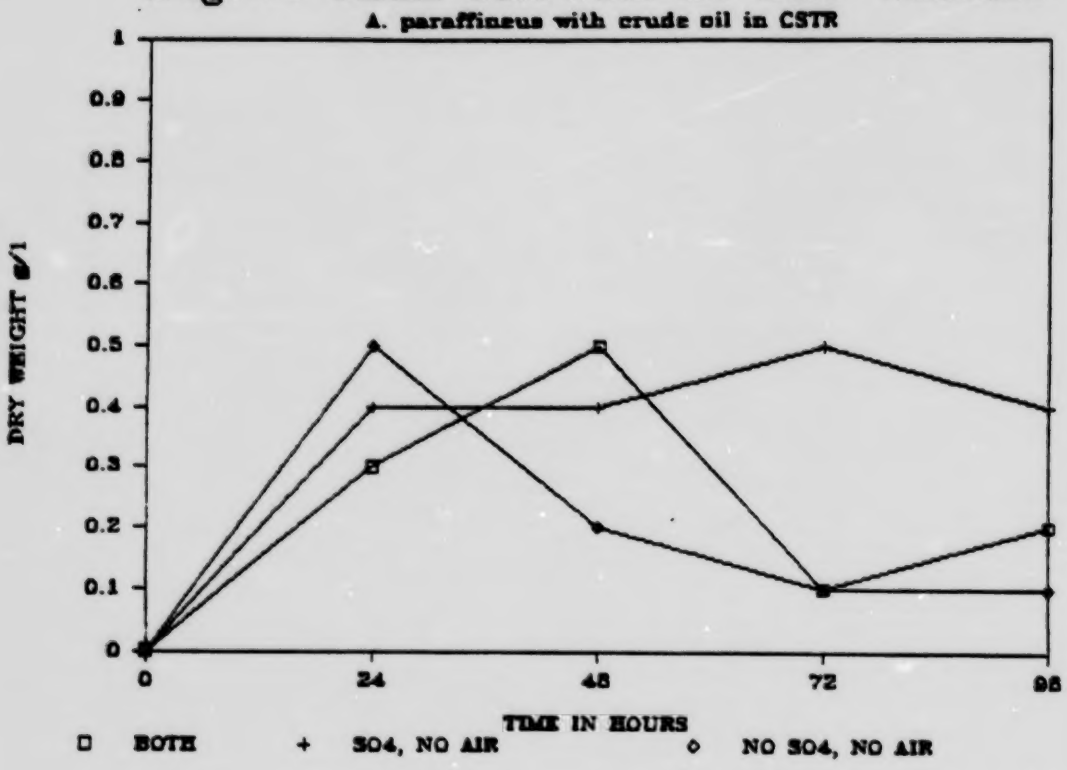


Fig. 11: CHANGE IN pH

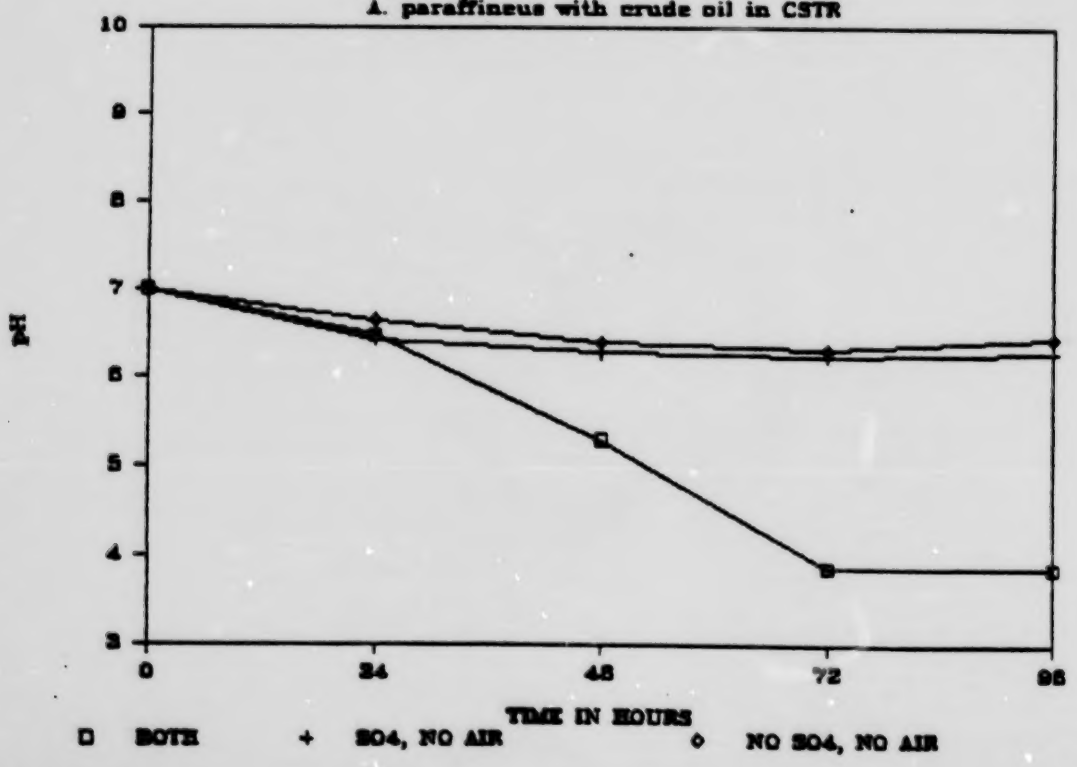


Fig. 12: PENTANE INSOLUBLES

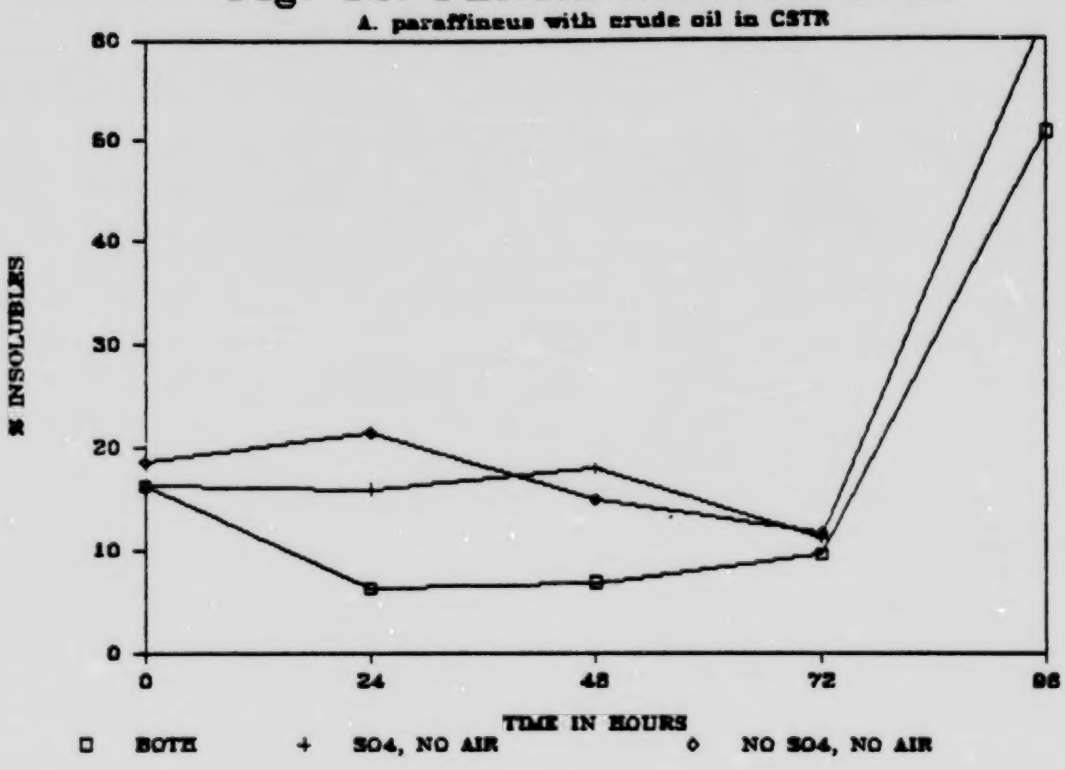


TABLE 8:

AIRLIFT FERMENTER
A. paraffineus with crude oil

PARAMETERS	INITIAL	24 HOURS	48 HOURS	72 HOURS
pH	7.00	7.03	7.15	7.20
TEMPERATURE C	30.0	25.0	25.0	24.0
DRY WEIGHT g/l	0.00	0.40	0.80	0.10
SULPHUR REMOVAL %	0.00	0.17	0.14	0.11
VISCOSITY CStokes	403.12	NA	NA	1103.23
SURFACE TENSION MEDIUM	70.50	60.70	60.90	61.10
INTERFACIAL TENSION	32.10	23.90	38.60	39.50
SURFACE TENSION OIL	37.90	35.70	36.40	37.50
PENTANE INSOLUBLES	17.02	11.86	11.81	NA

MEDIUM USED: MSM + SO4/2 + YE/2 (4 Litres)
OIL SOURCE: LLOYDMINSTER CRUDE (1 Litre)
AERATION RATE: 0.6 v/v
AGITATION RATE: 295 rpm
INOCULUM: 5%

TABLE 9:

AIRLIFT FERMENTER
CONTROL AERATED WITH NITROGEN

PARAMETERS	INITIAL	24 HOURS	48 HOURS
pH	7.00	6.58	7.31
TEMPERATURE C	30.0	25.0	25.0
DRY WEIGHT g/l	0.00	0.20	0.10
SULPHUR REMOVAL %	0.00	0.00	0.05
VISCOSITY CStokes	403.12	410.99	811.65
SURFACE TENSION MEDIUM	70.50	64.50	67.10
INTERFACIAL TENSION	32.10	22.90	33.30
SURFACE TENSION OIL	37.90	35.00	36.40
PENTANE INSOLUBLES	17.02	25.20	64.87

MEDIUM USED: MSM + SO4/2 + YE/2 (4 Litres)
OIL SOURCE: LLOYDMINSTER CRUDE (1 Litre)
AERATION RATE: 0.6 v/v
AGITATION RATE: 295 rpm
INOCULUM: 5%

TABLE 10:

Desulfovibrio desulfuricans in CSTR

PARAMETERS	INITIAL	24 HOURS	48 HOURS
pH	7.00	6.58	6.83
TEMPERATURE C	30.0	29.0	29.5
DRY WEIGHT g/l	0.00	0.30	0.40
SULPHUR REMOVAL %	0.00	0.00	0.01
VISCOSITY CStokes	403.12	1062.94	NA
SURFACE TENSION MEDIUM	70.50	73.40	67.30
INTERFACIAL TENSION	32.10	30.60	47.80
SURFACE TENSION OIL	37.90	35.90	52.10
PENTANE INSOLUBLES	17.02	NA	NA

MEDIUM USED; MSM+YE (1g/L), 4.0 Litres
OIL SOURCE: LLOYDMINSTER CRUDE (1.0 Litre)
AERATION RATE: 1.0 L/min NITROGEN (0.2 v/v)
AGITATION RATE: 295 rpm
INOCULUM: NONE FOR 24 hrs, then 1%

TABLE 11:
A. paraffineus with 10% crude in CSTR

PARAMETERS	INITIAL	24 HOURS	48 HOURS	72 HOURS	96 HOURS
pH	7.00	6.45	6.34	6.44	6.60
TEMPERATURE C	30.0	27.5	28.0	28.0	28.0
DRY WEIGHT g/l	0.00	0.80	0.40	0.60	0.80
SULPHUR REMOVAL %	0.00	0.04	0.07	0.08	0.07
VISCOSITY CStokes	457.59	343.43	563.00	138.50	501.07
SURFACE TENSION MEDIUM	70.50	64.40	61.00	58.90	58.80
INTERFACIAL TENSION	32.10	21.10	24.00	22.90	24.30
SURFACE TENSION OIL	37.90	34.60	35.50	33.80	34.90
PENTANE INSOLUBLES	17.02	16.19	12.63	11.42	14.74

PARAMETERS	120 HR	144HR	168 HR	192 HR	216 HR	240 HR
pH	6.65	6.70	6.64	6.63	6.58	6.52
TEMPERATURE C	28.0	28.0	28.5	28.5	28.5	29.0
DRY WEIGHT g/l	0.70	1.00	0.60	0.80	0.70	0.50
SULPHUR REMOVAL %	0.06	0.07	0.08	0.08	0.07	0.10
VISCOSITY CStokes	NA	NA	630.60	417.75	530.55	485.30
SURFACE TENSION MEDIUM	61.90	64.10	65.00	635.00	63.80	63.20
INTERFACIAL TENSION	23.00	NA	21.20	24.10	25.60	25.80
SURFACE TENSION OIL	34.90	NA	35.50	36.10	36.60	36.20
PENTANE INSOLUBLES	12.08	NA	16.34	11.96	12.20	10.24

MEDIUM USED: MSM + YE/2 (4.5 Litres)
OIL SOURCE: LLOYDMINSTER CRUDE (0.5 Litre)
AERATION RATE: 0.0 v/v (none)
AGITATION RATE: 295 rpm
INOCULUM: 5%

TABLE 12:
A. paraffineus with 20% crude in CSTR

PARAMETERS	INITIAL	24 HOURS	48 HOURS	72 HOURS	96 HOURS
pH	7.00	6.63	6.43	6.69	6.98
TEMPERATURE C	30.0	29.0	29.0	29.5	28.5
DRY WEIGHT g/l	0.00	0.30	0.60	1.00	0.60
SULPHUR REMOVAL %	0.00	0.28	0.14	0.15	0.16
VISCOSITY CStokes	403.12	392.97	493.19	541.60	585.82
SURFACE TENSION MEDIUM	70.50	67.20	64.00	64.90	63.10
INTERFACIAL TENSION	32.10	22.10	19.90	22.80	24.80
SURFACE TENSION OIL	37.90	33.50	30.30	32.70	32.40
PENTANE INSOLUBLES	12.56	17.02	9.56	14.35	21.45

PARAMETERS	120 HR	144 HR	168 HR	192 HR	216 HR	240 HR
pH	6.98	6.88	6.84	6.89	6.87	6.52
TEMPERATURE C	28.5	29.0	29.0	29.5	29.0	29.0
DRY WEIGHT g/l	0.50	0.30	0.50	0.50	0.30	0.30
SULPHUR REMOVAL %	0.19	0.30	0.18	0.24	0.19	0.25
VISCOSITY CStokes	647.75	629.43	748.79	789.33	892.92	537.10
SURFACE TENSION MEDIUM	66.80	64.60	64.60	65.30	65.00	66.00
INTERFACIAL TENSION	32.80	31.30	21.70	23.70	24.10	23.10
SURFACE TENSION OIL	37.80	33.90	36.20	33.60	36.70	33.30
PENTANE INSOLUBLES	32.54	12.41	10.2	29.26	13.86	NA

SMHYE 1g/L (4.0 litres)
L SOURCE: LLOYDMINSTER CRUDE (1.0 litre)
ERATION RATE: none
ITIATION RATE: 295 rpm
OCCULUM: 10 %

TABLE 13:
A. paraffineus with soln. of DBT in paraffin in CSTR

HOURS	0	24	48	72	96
pH	7.32	7.26	6.83	5.50	5.40
TEMPERATURE C	30.0	28.0	30.0	29.5	29.0
OD @ 620 nM	0.13	0.16	0.18	0.11	0.07
SO4 ABS @ 420 nM	0.16	0.09	0.22	0.16	0.27
VISCOSITY CStokes	84.70	90.30	76.50	88.60	89.60
SURFACE TENSION MEDIUM	58.10	41.80	69.70	61.10	61.60
INTERFACIAL TENSION	28.90	17.00	23.90	8.90	20.90
SURFACE TENSION OIL	34.70	31.90	30.20	33.40	33.90

MEDIUM USED: MSM + SO4 + YE (.25 g/L)
OIL SOURCE: PARAFFIN/DBT SATURATED SOLN (1 L)
AERATION RATE: 0.6 v/v
AGITATION RATE: 295 rpm
INOCULUM: 15%

Fig. 13: INTERFACIAL TENS. 10–20% CRUDE

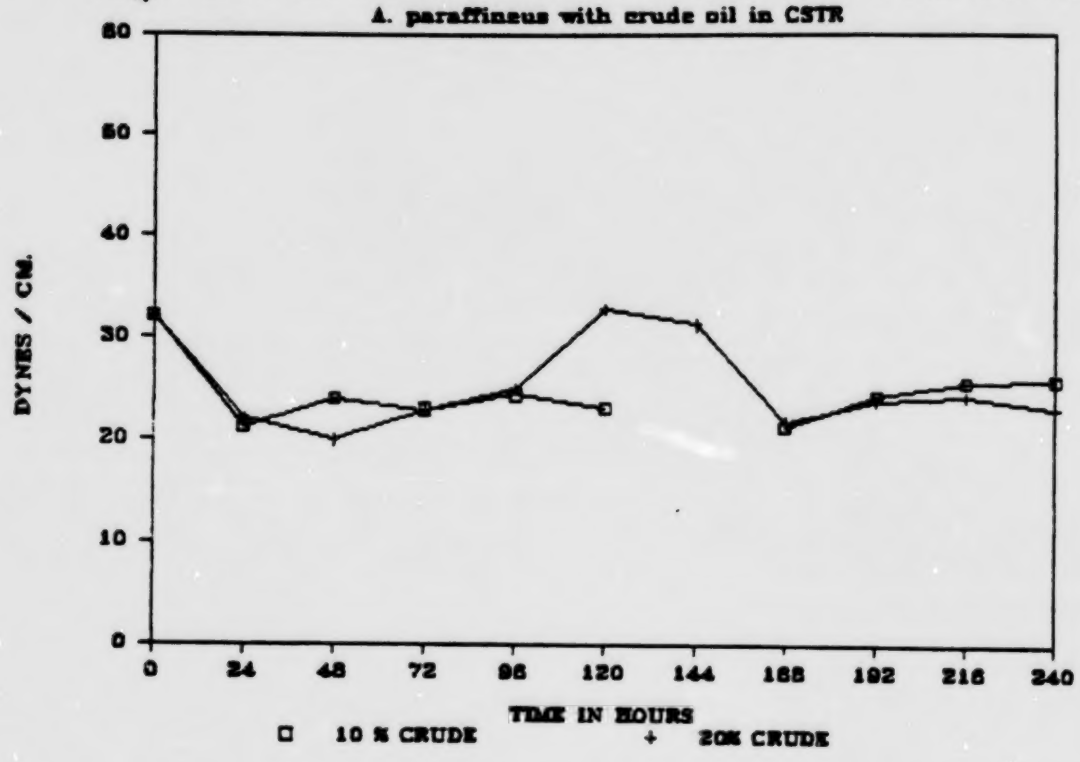


Fig. 14: OIL VISCOSITY, 10–20% CRUDE

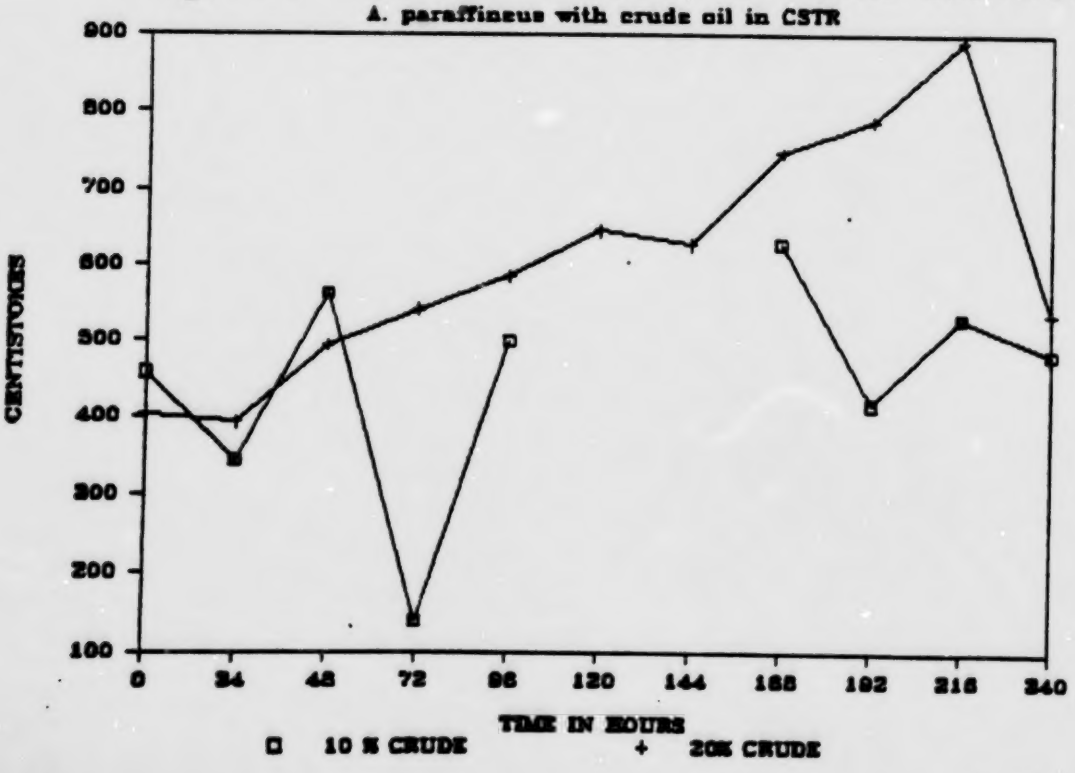


Fig. 15: OIL SURF TENS. 10–20% CRUDE

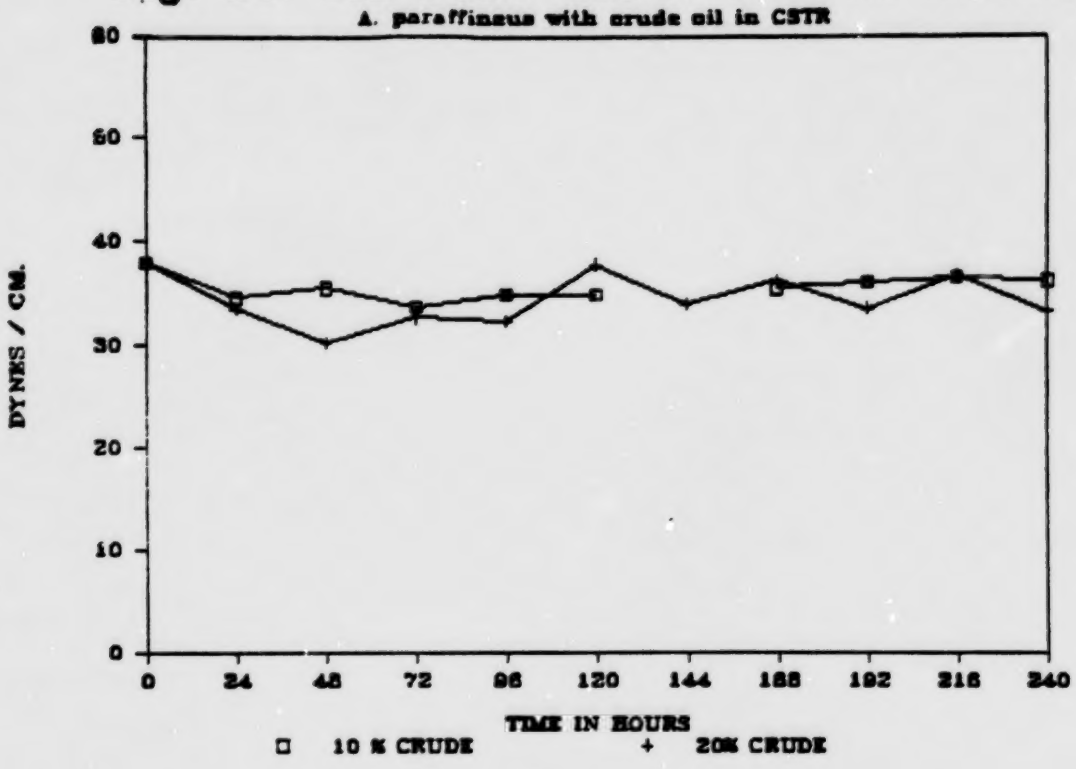


Fig. 16: % S REMOVAL 10–20% CRUDE

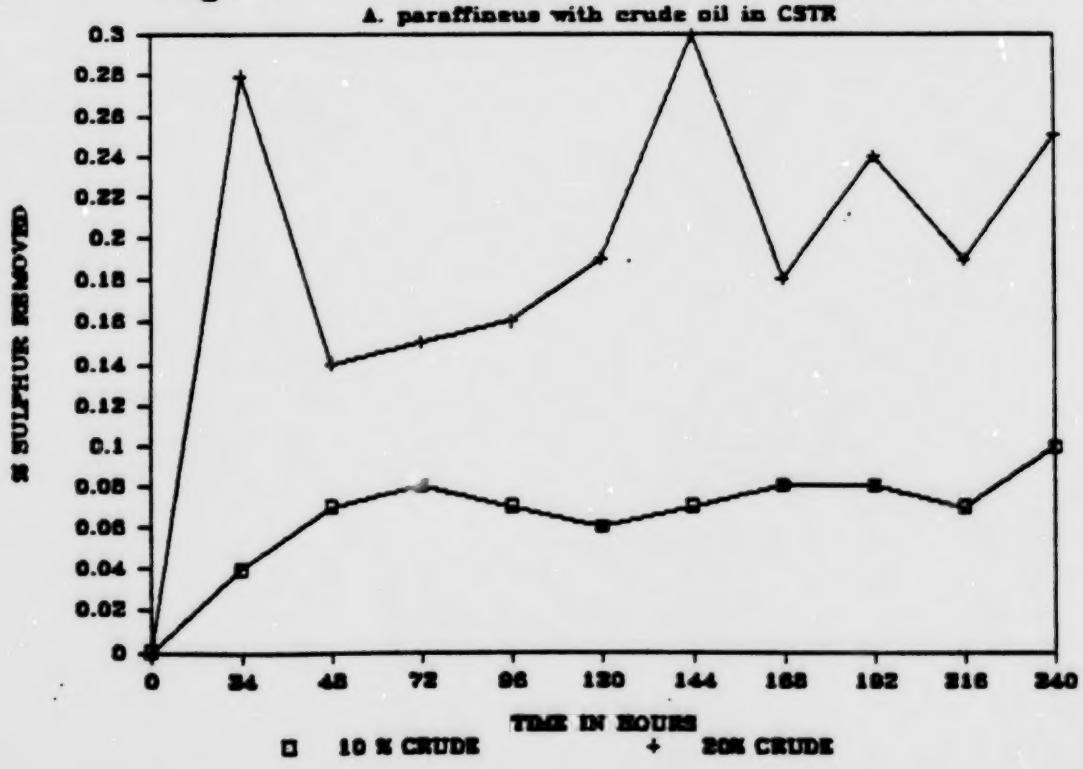


Fig. 17: SO4 CONC (ABS 420 NM)

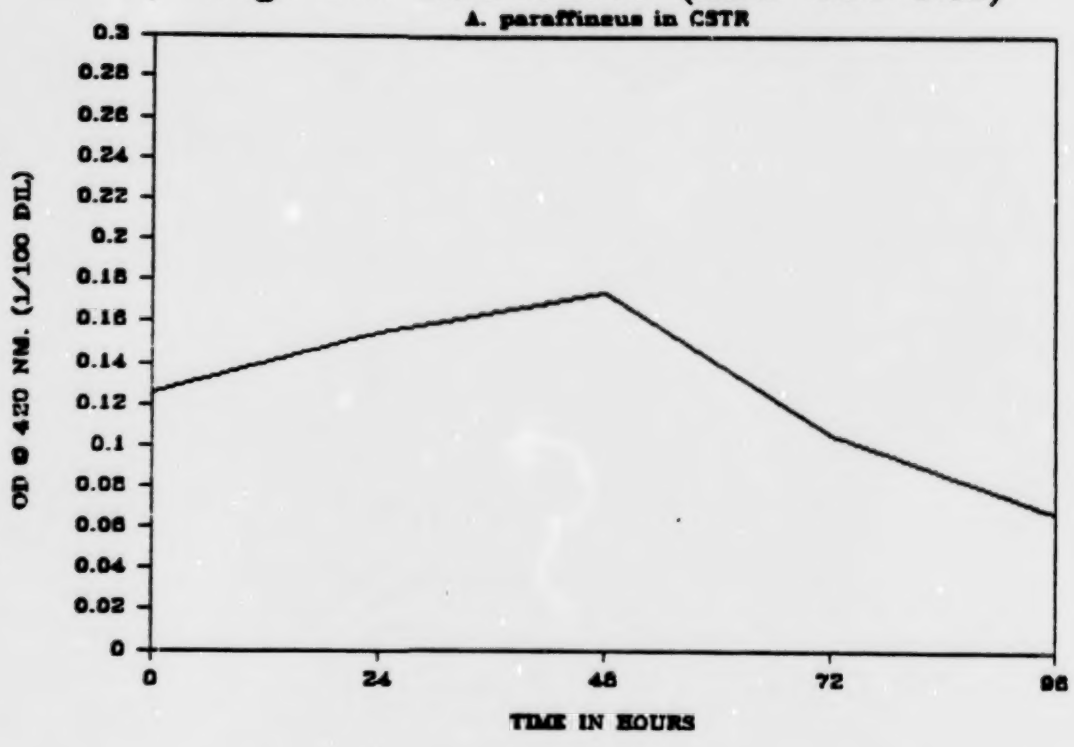


Fig. 18: OIL SURFACE TENSION

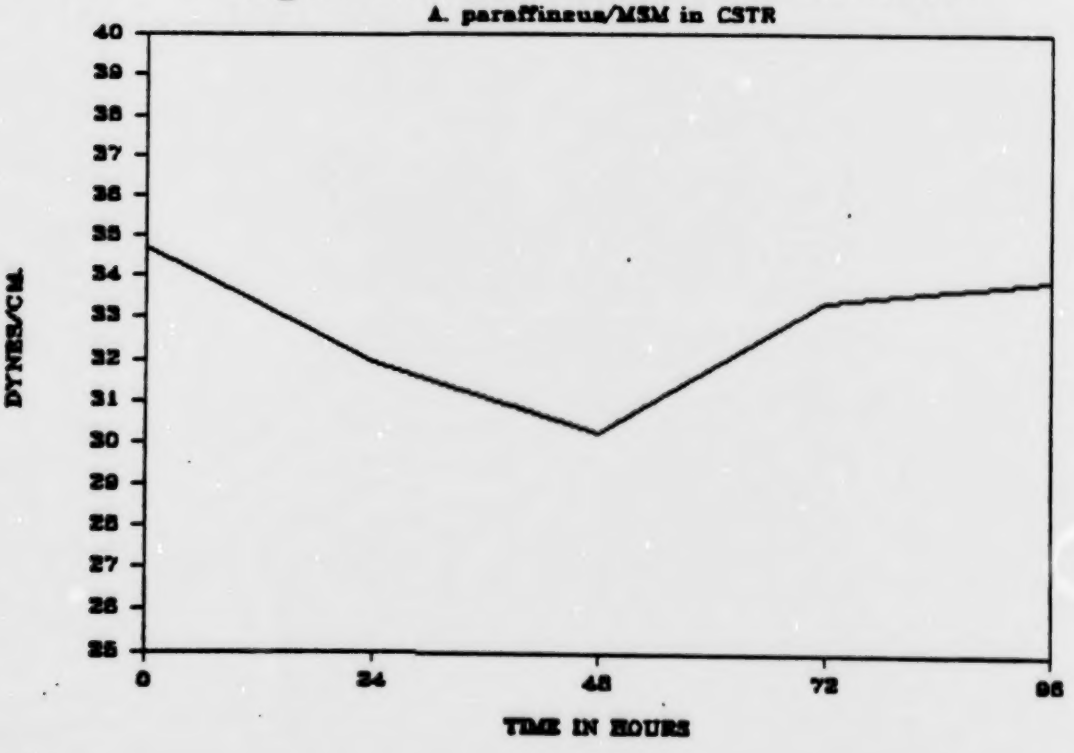


Fig. 19: MEDIUM SURFACE TENSION

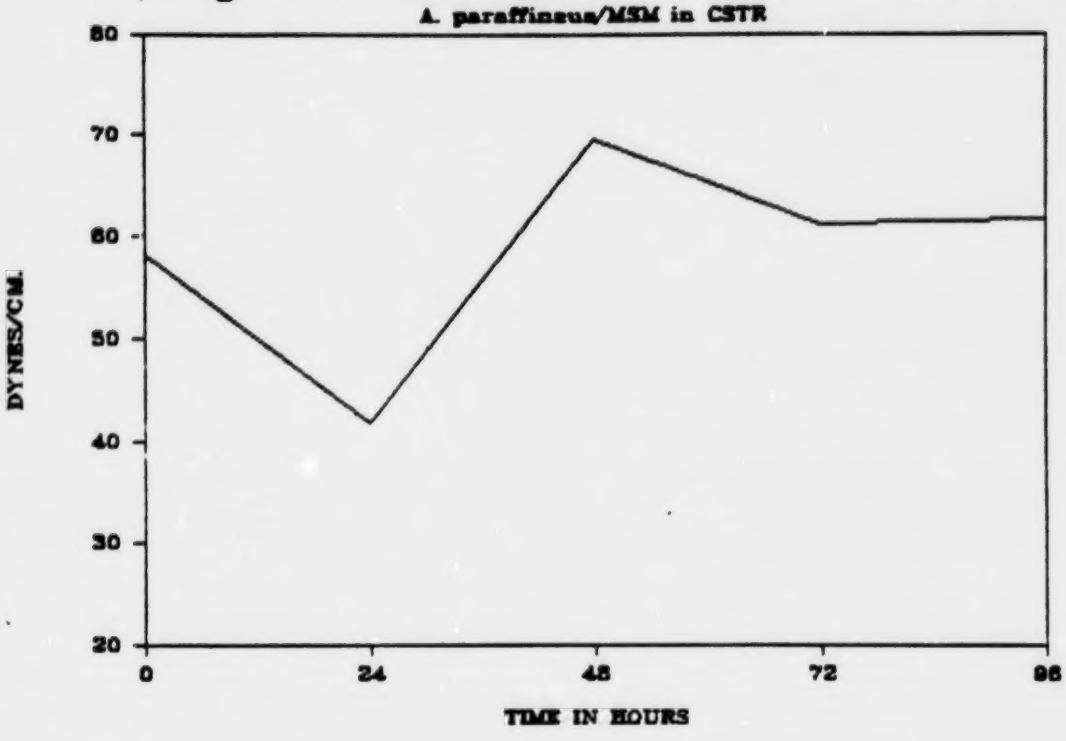


Fig. 20: INTERFACIAL TENSION

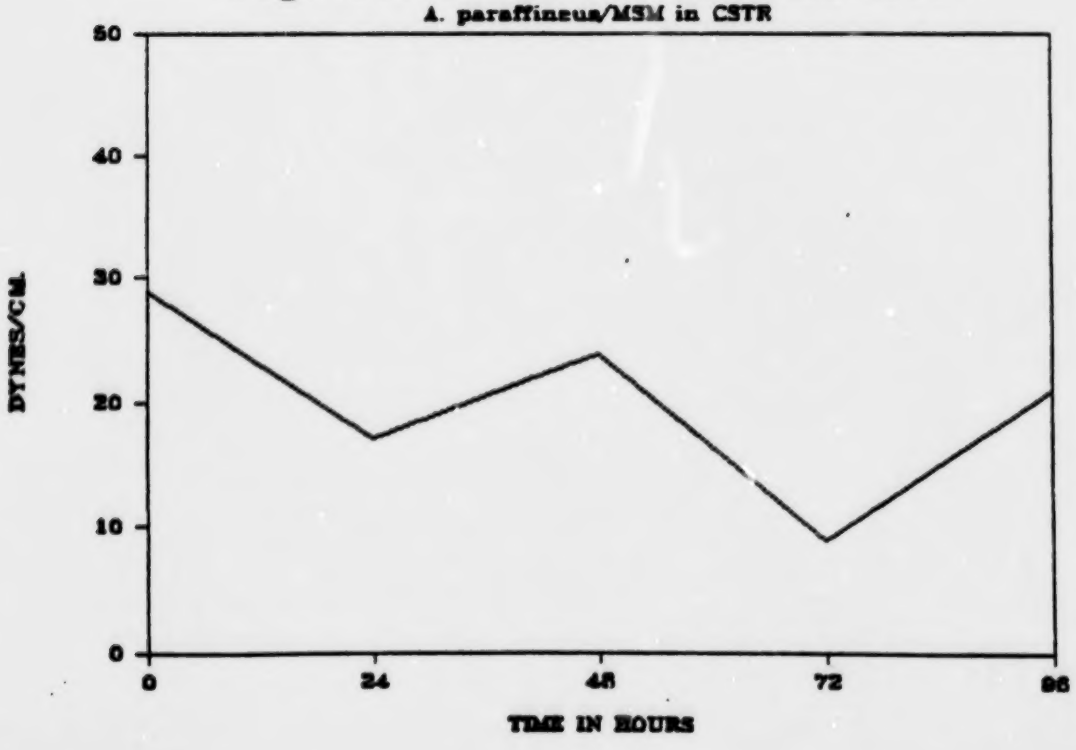


Fig. 21: PARAFFIN OIL / DBT VISCOSITY

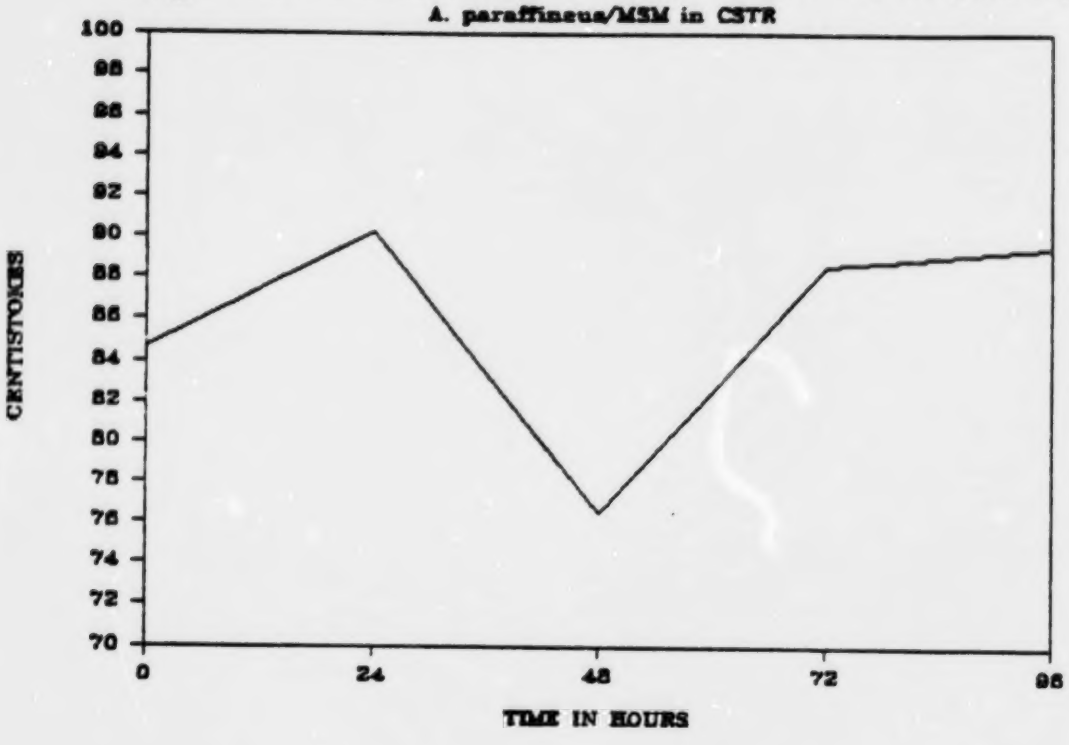
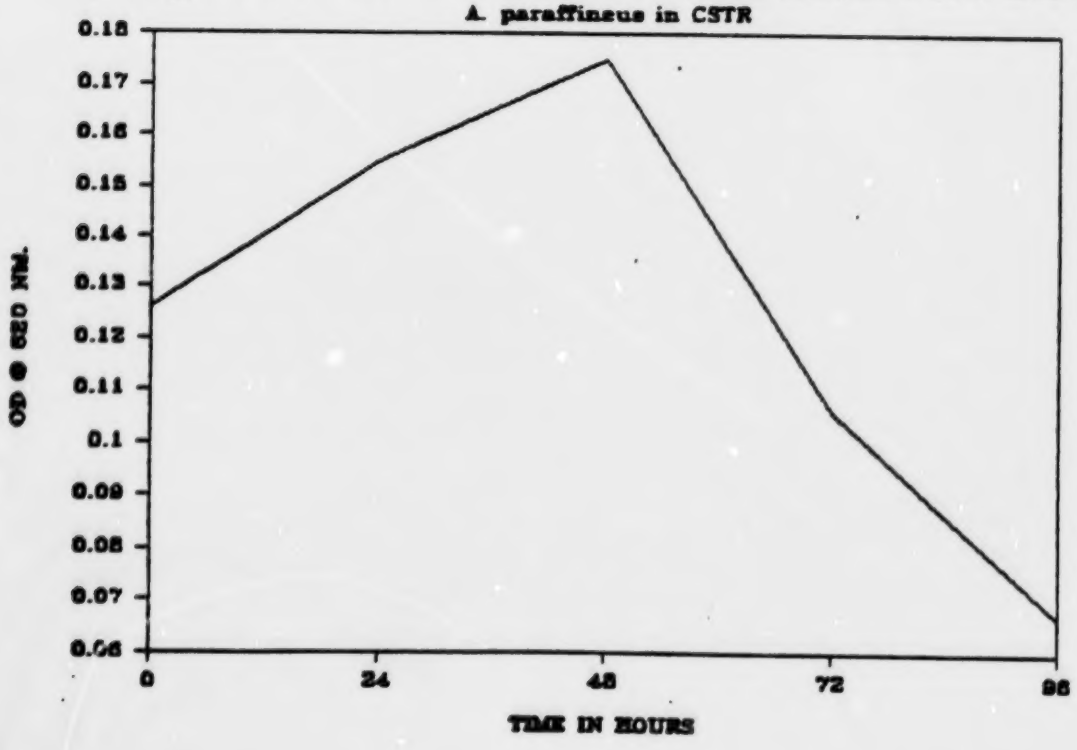


Fig. 22: MEDIUM OD, 20% PARAFFIN/DBT



DISCUSSION

Most earlier studies of microbial desulphurization of oils employed synthetic substrates, particularly paraffin solutions of dibenzothiophene (DBT), as the model sulphur containing compounds. The efficiency of microbial desulphurization in synthetic media has been in excess of 80% (Eckart et al., 1980; Finnerty et al. 1983; Finnerty and Robinson, 1986; Kargi and Robinson, 1984). In contrast, only a handful of studies addressed the removal of sulphur from heavy oils. The current study has proven that the bacteria Arthrobacter paraffineus, Pseudomonas putida, Pseudomonas aeruginosa, and to a lesser extent Pseudomonas alcaligenes and Desulfovibrio desulfuricans are able to remove sulphur from crude oil. The extent of sulphur removal, however, was substantially and consistently less than that reported for synthetic medium. In the present study, maximum sulphur removal efficiency after 6-day incubation period was 21.4% with Arthrobacter paraffineus, 18.5% with Pseudomonas putida and 18.3% with Pseudomonas aeruginosa at 30°C in aqueous suspensions containing 15% (volume basis) Lloydminster crude oil in shake-flask cultures. In the 30L air-lift and 6.5L continuous stirred tank reactors the sulphur removal efficiency was consistently less. In continuous, stirred tank reactors, maximum sulphur removal from crude oil was 11.8% with Arthrobacter paraffineus cultures after 6 days of incubation. The air-lift fermentor design was not suitable for microbial desulphurization, since the high aeration (0.5 to 1.0 m^3/m^3 medium-minutes) required to produce sufficient mixing has resulted in unwanted oxidation of the oil fraction. This is reflected by the large increases of oil viscosity, toluene insoluble and

pentane insoluble fractions. The oil became lighter in colour and became gummy in consistency.

The microorganisms, Desulfovibrio desulfuricans was capable of removing sulphur as dissolved sulphides from synthetic media containing DBT under anaerobic conditions. Sulphur removal after 9 days of incubation was 15% in paraffin-DBT and 2.3% in dodecane-DBT medium. However, in cultures containing 15% crude oil, sulphur removal was insignificant. It appears that some components of crude oil inhibited the growth of Desulfovibrio desulfuricans.

In all cultures, sulphur removal from the oil fraction was non-growth associated. Most desulphurization occurred in stationary cultures after growth was essentially completed. This apparent independence of sulphur metabolism from growth has been noted previously (Finnerly and Robinson, 1986). The nutritional requirements of microorganisms were variable. The bacteria Arthrobacter paraffineus, Pseudomonas putida and Pseudomonas aeruginosa utilized some components of the crude oil as carbon and energy sources. The bacterium, Arthrobacter paraffineus had an absolute requirement, while Pseudomonas putida and Pseudomonas aeruginosa benefited from the supply of yeast extract in the medium. Presumably, Arthrobacter paraffineus requires some vitamin B complex as a growth factor. The bacterium, Pseudomonas alcaligenes required the presence of citrate and/or lactate for both growth and sulphur metabolism. The bacterium Desulfovibrio desulfuricans benefited from the use of a reducing medium containing thioglycollate. Sulphur removal from crude oil was significantly less than from synthetic solutions. Samples of oil

emulsions, submitted to CANMET for sulphur analysis, indicated minimal sulphur reduction in the oil phase in Arthrobacter paraffineus cultures. The presence of sulphate in the medium may have had a significant beneficial effect on the desulphurization of crude oil by Arthrobacter paraffineus, Pseudomonas putida and Pseudomonas aeruginosa. It follows that an optimal sulphate concentration exists, which may be species dependent. This optimal sulphate content is yet to be determined. In general, the sulphur removal efficiency was highly dependent on the media and further studies on the optimization of the medium and the environmental conditions are warranted.

The experimental results were highly variable. This variability reflects the difficulties of experimenting with highly coloured, viscous material, such as crude oil and the use of traditional bacteriological and biochemical methodology that are more suitable for different conditions. Due to this variability in the data, the experimental results were subjected to extensive statistical analysis using the SAS package of the Department of Computing Services, University of Waterloo. The results of the statistical analysis are summarized in Table 13 and discussed in more detail below.

Sulphur removal by various species has been compared for statistical difference using Analysis of Variance and Student Newman-Keuls Test. Although Arthrobacter paraffineus performed slightly better than other species, the difference in sulphur removal between Arthrobacter paraffineus, Pseudomonas putida and Pseudomonas aeruginosa (see Table 13)

are statistically not significant. The mean sulphur removal in 6-day incubation was 7.9% at 30°C and 6.7% at 20°C. Three temperatures (25°C, 30°C and 37°C) have been compared for sulphur removal by Arthrobacter paraffineus from 15% (volume basis) crude oil under various medium and operating conditions. Comparing the sulphur removal at 20°C and 30°C using Arthrobacter paraffineus, a slight statistical significance (= 0.07) was noted. The sulphur removal efficiency at 30°C averaged 11.1% in comparison to 4% at 25°C. Table 1 compares the sulphur removal for various species at 25°C, 30°C and 37°C. No significant difference was noted between these temperatures. The mean percent sulphur removal was 7.9% at 30°C, 6.7% at 25°C and 2.2% at 37°C. It appears that 30°C is a near-optimum temperature for desulphurization with the bacterial species employed in this work.

TABLE 14: Dependent Variables

	BIOMASS CONCENTRATION	SULFUR	ST_{med}	ST_{oil}	IFT	VISC.	PENT	TOLUENE
HC source		++						
Medium								
Table 1		NS						
Table 10		++	NS	++	++	NS	NS	NS
Table 14		++	NS	NS	NS	NS	++	NS
Temperature								
Table 10		+	NS	+	NS	NS	NS	NS
Table 13		NS	NS	NS	NS	NS	NS	NS
Yeast Extract		NS	NS	++	++	NS	NS	NS
Organism		NS	NS	NS	NS	NS	+	NS
Aeration								
MSM + SO_4	NS		NS	++	++	NS	NS	
MSM	NS	NS	NS	NS	++	+	NS	

ST_{med} = surface tension medium
ST_{oil} = surface tension oil
NS = not significant
++ = significant to = 0.05 level
+ = significant to = 0.05 - 0.15 level

Experimental data with Desulfovibrio desulfuricans was used to compare DBT in dodecane, DBT in paraffin and crude oil for sulphur removal. Desulphurization from paraffin solutions was significantly higher (= 0.0001) than from dodecane solutions and crude oil. The means were 10.7% sulfur removal from paraffin, 2.9% from dodecane and 0.17% sulphur removal from crude oil.

As indicated above, the presence of sulphate in the medium was beneficial on the desulphurization efficiency. The overall mean desulphurization efficiency with sulphate containing media was 13.8% as compared to 0.51% without sulphur. The difference was proven to be statistically highly significant.

Desulphurizing bacteria are known to produce surfactants. These surface active agents are often integral components of the cell wall and the cytoplasmic membrane. They can affect the rheological properties of both the water and the oil phases. For example, the air/water surface tension of 72 dynes cm^{-1} can decline significantly by the natural surfactants to 30-40 dynes cm^{-1}. (Any reduction below 20 dynes cm^{-1} is rare). An important aspect of these surfactants is the emulsification of the oil fraction. Many biosurfactants are known to be excellent emulsifiers. This emulsification results in surface area, hence, higher bacterial activity. The oil/water interfacial tension is a good measure of this biological activity. The interfacial surface tension between crude oil and water is 42 dynes cm^{-1}. Biological activity resulted in the reduction of the interfacial surface tension to 16.0 dynes cm^{-1} and 29.0 dynes cm^{-1} in media with and without sulphate after 6 days incubation. The

surface tension of the oil phase also declined significantly to mean values of 45.4 dynes cm^{-1} and 41.0 dynes cm^{-1} media with and without sulphate when desulphurization was significant. In contrast, the viscosity of the oil was not affected significantly. It is noteworthy that the three most active organisms, Arthrobacter paraffineus, Pseudomonas putida and Pseudomonas putida showed insignificant differences as they affected the aqueous, the oil, and the interfacial surface tensions.

An important aspect of the study was the assessment of the potential effect of bacterial action on crude oil quality. This was assessed by determining the pentane insoluble and the toluene insoluble fractions of the crude oil. The pentane insoluble fraction is known as the asphaltene content. It is composed of complex, polyaromatic compounds with high carbon to hydrogen ratio. Some of these compounds may be partly oxidized. The toluene insoluble fraction represents the partially oxidized petroleum fraction that may result from biological or non-biological oxidative processes. Both the pentane and toluene insoluble fractions were highly variable, but generally an upward trend with incubation was noted. The conditions of desulphurization had no significant effect on the toluene insoluble fraction.

In contrast, the pentane insoluble fraction was dependent on growth conditions. The mean pentane insolubles averaged lower (11.3%) in media where active desulphurization (with sulphate) had taken place and somewhat higher (12.3%) in media lacking sulphate.

The studies reported above were performed with monocultures. Recent studies (Eckart et al, 1986) have indicated that mixed cultures, particularly mixed anaerobic cultures have performed significantly better than pure cultures. More than 40% desulphurization of crude oil has been reported with heterogeneous anaerobic populations in 2-3 days. The use of elective, mixed cultures may, in fact, be a more promising approach for developing a biotechnology-based desulphurization process. It is clear, however, that substantial amount of further research is necessary.

CONCLUSIONS AND RECOMMENDATIONS

The major conclusions and recommendations of the present study may be summarized as follows:

CONCLUSIONS

1. The bacterial species Arthrobacter paraffineus, Pseudomonas putida, Pseudomonas alcaligenes and Pseudomonas aeruginosa were capable of removing sulphur from Lloydminster crude oil under aerobic conditions. Sulphur removal by Desulfovibrio desulfuricans under anaerobic conditions was limited due to the apparent growth inhibitory effect of the crude oil. Maximum sulphur removal after 6 days of incubation was 21.4% with Arthrobacter paraffineus, 18.5% with Pseudomonas putida and 18.3% with Pseudomonas aeruginosa cultures in aqueous suspensions containing 15% (volume-basis) Lloydminster crude. Sulphur metabolism was non-growth associated and continued in stationary phase cultures.

2. Medium and growth conditions greatly influenced sulphur removal. The presence of sulphate in the medium was particularly beneficial. At 30°C, the sulphur removal efficiency was slightly better than at lower temperatures. Arthrobacter paraffineus and Pseudomonas putida utilized crude oil as sole source of carbon. The bacterium Arthrobacter paraffineus benefited by the supply of vitamins in the medium. The bacterium, Pseudomonas alcaligenes required complex media containing either citrate or lactate for growth.

3. The bacterium, Arthrobacter paraffineus was proven to be slightly better with respect to desulphurization activity and reduction of the interfacial surface tension than other species. For this reason, this was selected for fermentor studies.

4. Air-lift reactors are not suitable for bacterial desulphurization. The aeration rates required for adequate mixing caused a significant deterioration of the crude oil in terms of increased viscosity and increased pentane insoluble and toluene insoluble fractions. The stirred tank configuration with low aeration ($0.06 m^3/m^3.min$) was proven to be better inasmuch as 11.8% average sulphur removal was realized after 6 days of incubation.

RECOMMENDATIONS

The rate of bacterial desulphurization in crude oil, although significantly lower than in synthetic solutions, provides impetus and encouragement for further study. It is recommended that the following research topics are examined in more detail.

1. The bacterial desulphurization activity of the genetically engineered strains of Pseudomonas alcaligenes should be evaluated with crude oil as the substrate. These organisms have been reported to possess exceptionally high desulphurization activity with synthetic substrates.

2. Anaerobic desulphurization studies with mixed cultures are recommended. Anaerobic conditions minimize oil degradation by partial oxidation.

3. Further studies are required to establish optimal medium and environmental conditions under both aerobic and anaerobic conditions. Under aerobic conditions, the optimal aeration rate remains to be established.

4. It is evident from this study that conventional bioreactor designs such as pneumatically mixed vessels and stirred tank reactors are not well suited for bacterial desulphurization. Therefore, a very important research area is the development of bioreactors that would provide good contact between the oil phase and the bacteria while the deterioration of the crude oil is minimized. The scale-up potential of these specifically designed bioreactors should be examined.

5. The applicability of immobilized whole cell bioreactors for continuous, high rate desulphurization should be investigated. Immobilized cell cultures, similar to anaerobic fixed film reactors, would provide high bacterial densities for rapid sulphur removal. These types of reactors have been proven to be highly efficient for treating industrial wastewater in Europe.

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APPENDIX I

MATERIALS AND METHODS

(a) Continuously Stirred Tank Reactor Studies

CSTR studies were carried out with a modified 5 litre New Brunswick Scientific fermentor (New Brunswick Scientific Co., Edison, N.J.). Air was taken from the laboratory supply and passed through an inline filter. The fermentor temperature was regulated using a temperature controller (YSI Thermistep, Model 71).

Prior to each run, the assembled fermentor was filled with medium and autoclaved for 75 minutes at 121°. After cooling the substrate was added and the inoculum introduced. Inoculum consisted of 5-15% of medium volume of cells growing in log phase in the medium used in the reactor. The mixture was stirred at 295 rpm and aerated at a chosen rate. Initially, pH was monitored with a probe fixed inside the fermentor. When oil fouling of the probe became a problem, pH measurements were taken from aqueous phase samples on the bench.

The fermentor was fitted with sampling tubes having threaded plugs and extending to the depth of the oil and aqueous phases. When sampling, agitation was stopped and the plugs were replaced with 40 or 60 mL disposable syringes (Becton Dickenson, Rutherford, N.J.) and samples withdrawn and placed into disposable tubes. Synthetic oil samples were centrifuged 30 min. at 2000 rpm (g) to separate oil, water and cells.

(b) Shake Flask Studies

The inocula were grown in glass bacteriological culture tubes, set in racks and incubated at the desired temperature. Tubes were partially filled with medium and inoculated with a loop of cells from a slant maintenance culture, using appropriate sterile technique. Oil was then introduced to the tube and a plastic or metal cap put on. Agitation was performed by rocking the tubes gently once a day. After the desired incubation period, the contents of the test tubes were transferred into 250 ml shake flask containing 100 ml liquid (15% to 30% oil phase). The shake flasks were placed on a rotary shaker (60 rpm) and incubated at the desired temperature.

APPENDIX II

ANALYTICAL TECHNIQUES

(a) Sulphate Determination

The method used to determine sulphate concentration in the medium was adapted from Standard Methods for the Examination of Water and Wastewater, 14th Ed., p. 496, American Public Health Association, Washington, 1979. The following alterations were made to the published method. The oil and water layers were allowed to separate. The water layer was then filtered through an 0.2 mu filter.

A 10% w/v solution of barium chloride solution was used, 2 mL being added to 10 mL of sample and 0.5 mL of conditioning reagent to initiate the reaction.

A PYE Unicam Model SP6-550 UV/VIS spectrometer was used to make absorbance measurements at a wavelength of 420 nM.

Sulphate concentration was calculated using the formula:

$$C = K \times ABS + B$$

where K for crude oil is 152.85 and B is 6.2292.

(b) Sulfide Determination

The method used was also adapted from Standard Methods for Examination of Water and Wastewater, 14th Ed., p. 499, with the following alterations:

A standard curve was prepared using a series of known concentrations of potassium sulphide and used to make quantitative analyses of the test samples. Absorbance of standard and test samples were measured at 440 nM using a PYE Unicam Model SP6-550 UV/VIS spectrophotometer.

(c) Surface Tension Measurements

Surface and interfacial tension measurements were made with a Fisher Surface Tensiomat Model 21 (Fisher Scientific Co., Nepean, Ontario) according to the manufacturer's instructions.

(d) Viscosity Measurements

Viscosity measurements were made with a Cannon Capillary viscometer (Cannon Instrument Co., State College, Pa.) used at 40° according to the instructions supplied by the manufacturer.

(e) Pentane and Toluene Insolubles Determination

The percentage of pentane and toluene insolubles present was determined by an in-house method of CANMET Energy Research Labs which was itself adapted from the ASTM D-20420-81 Assay.

Procedure:

A thoroughly mixed sample (1 to 1.5 g) was added to an accurately weighed 125 mL Erlenmeyer flask and reweighed. Solvent (100mL) was added, stoppered and shook until all the sample was in suspension. The flask was placed in a sonic bath for 15 minutes, removed and left at room temperature overnight.

A clean gooch crucible was accurately weighed with an inserted filter pad. The contents of the Erlenmeyer flask were run through the crucible using light suction. The flask was washed thoroughly with clean solvent.

The flask and crucible were placed in a drying oven at 120°C for one hour. They were brought to room temperature and reweighed.

The percent insolubles are calculated as follows:

$$\% \text{ insolubles} = \frac{\text{weight of insolubles in flask} + \text{weight of insoluble in crucible}}{\text{weight of sample}}$$

(f) Dry Weight

The dry weight of the culture was determined by filtering 20 ml solution through a pre-weighed millipore filter at 0.2 micron millipore filter. The filter and the biomass were dried at 105°C for 12 hours.

APPENDIX III

(a) Cultures

Arthrobacter paraffineus ATCC #19558 maintenance cultures were grown on nutrient agar slants at 30° with monthly transfers. They were stored at 4°.

(b) Media

See attached.

Medium for the Growth of Pseudomonas alcaligenes

CITRATE

Na_2HPO_4	1.25g
K_2HPO_4	1.4g
$CaCl_2$	0.076g
$MgCl_2.6H_2O$	0.25g
$MnCl_2.4H_2O$	0.01g
NH_4NO_3	2.0g
$FeCl_2.4H_2O$	0.016g
Yeast Extract	1.0g
Sodium Citrate	10.0g

per 1 litre deionized water

CITRATE Variation

as for Citrate, except

Yeast Extract	0.5g
Sodium Citrate	5.0g

LACTATE

as for Citrate, except

Yeast Extract	1.0g
Sodium Lactate	10.0ml

LACTATE Variation

as for Lactate, except

Yeast Extract	0.5g
Sodium Lactate	5.0ml

LACTATE and CITRATE (L & C)

Na_2HPO_4	1.25g
K_2HPO_4	1.4g
$CaCl_2$	0.076g
$MgCl_2.6H_2O$	0.25g
$MnCl_2.4H_2O$	0.01g
NH_4NO_3	2.0g
$FeCl_2.4H_2O$	0.016g
Yeast Extract	1.0g
Sodium Citrate	5.0g
Sodium Lactate	5.0g

per 1 litre deionized water

L & C Variation

as for L & C, except

Yeast Extract	0.5g
Sodium Citrate	2.5g
Sodium Lactate	2.5g

MINERAL SALTS MEDIA

for the Growth of Pseudomonas aeruginosa, Pseudomonas putida, Rhodococcus erythropolis, Arthrobacter paraffineus.

BASAL MINERAL SALTS MEDIUM (MSM)

NH_4NO_3	2.6g
KH_2PO_4	0.6g
$MgCl_2.6H_2O$	0.46g
$CaCl_2$	0.11g

per 1 litre deionized water

VARIATIONS

MSM + YE

as for MSM, adding

Yeast Extract	1.0g
or	
Yeast Extract	0.5g
or	
Yeast Extract	0.25g
or	
Yeast Extract	0.1g
or	
Yeast Extract	0.05g
or	
Yeast Extract	0.01g

(per 1 litre of MSM)

MSM + YE + GL

as for MSM, adding

Yeast Extract	1.0g
Glucose	10.0g

MINERAL SALTS MEDIUM with SULPHATE (MSM + SO4)

$(NH_4)_2SO_4$	2.6g
KH_2PO_4	0.56g
$CaCl_2$	0.11g
$MgSO_4.7H_2O$	0.5g
$FeCl_3.6H_2O$	0.04g

per 1 litre deionized water

MSM + SO4 + YE

as for MSM + SO4, adding

Yeast Extract	1.0g
or	
Yeast Extract	0.5g
Yeast Extract	0.25g
or	
Yeast Extract	0.05g
or	
Yeast Extract	0.01g

per 1 litre deionized water

MSM + SO4 + YE + GL

as for MSM + SO4, adding

Yeast Extract	1.0g
Glucose	10.0g

MSM for the Growth of <u>Sulfolobus acidocaldarius</u>

MSM

NH_4NO_3	2.6g
KH_2PO_4	0.6g
$MgCl_2.6H_2O$	0.46g
$CaCl_2$	0.11g

per 1 litre deionized water
pH range 2.0-3.0

MSM + S

as for MSM, adding

Elemental Sulphur	10.0g

MSM + YE + S

as for MSM + S, adding

Yeast Extract	1.0g

MSM + CuS

as for MSM, adding
Cupric Sulphide 10.0g

MSM + YE + CuS

as for MSM + CuS, adding
Yeast Extract 1.0g

ATCC 1256 (modified) for S. acidocaldarius

(NH4)2SO4	1.3g
KH2PO4	0.28g
MgSO4.7H2O	0.25g
CaCl2	0.07g
FeCl3.6H2O	0.02g
MnCl2.4H2O	1.8mg
Na2B4O7.10H2O	4.5mg
ZnSO4.7H2O	0.22mg
Yeast Extract	1.0g

per 1 litre deionized water

API BROTH for SULPHATE REDUCERS

Yeast Extract	1.00g
Ascorbic Acid	0.10g
MgSO4	0.20g
K2HPO4	0.01g
Ferrous Ammonium Sulphate	0.10g
NaCl	10.00g

per 1 litre distilled water
final pH ~7.4
Sodium Lactate 4.0ml

API AGAR

as for API Broth, adding
Agar 14.00g

API without SULPHATE

as for API Broth, excepting
MgSO4 0.0g

THIOGLYCOLLATE BROTH (DIFCO-BACTO)

Bacto-Yeast Extract	5.0g
Bacto-Casitone	15.0g
Bacto-Dextrose	5.0g
L-Cystine	0.25g
NaCl	2.5g
Sodium Thioglycollate	0.5g
Bacto-Agar	0.75g

per 1 litre deionized water

APPENDIX IV

RAW DATA

EXPERIMENT: 2 Fermentor Aeration 3L/min

BEGIN DATE: 870525
END DATE: 8705

FLASK LABELS alpha / numeric

RANGE: 0 h - 24/48/72/96 hr

SPECIES: A. paraffineus

MEDIUM USED: MSM + 1/2 SO_4 + 0.5 g YE/L

INITIAL pH: 7.00

TEMPERATURE: 29.5

HYDROCARBON SOURCE: Lloydminster Crude 20% vol.

PERSONS INVOLVED: Norah Davidson
Peter Daugulis

COMPUTER INFORMATION:

FERMENTER

EXPERIMENT # 2

SPECIES: A. paraffineus

DATE: 870525

APPARATUS: NB Scientific 7.5L

INITIAL CONDITIONS: pH 7.0; Temp 30°C; 1L crude oil Lloydminster MSM + 1/2 SO_4 + .5 g/L YE 4 litres. 250 ml seed aeration 3L/min (.6 v/v)

PARAMETERS	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH	6.48	5.29	3.86	3.86	
TEMP	30.5	30.0	30.5	29.0	
OD @620	.257	.447	.195	.087	
DRY WT	.3	.5	.1	.2	
SUBJ.-AQ	++		++	++	
SUBJ.-OIL	-	-	--	--	
VOL AQ HARVESTED	150 ml	150ml	150ml	150ml	
VOL OIL HARVESTED					
SO4 ABS @420 .187 1/50 DIL	.061	.094	.085	.100	
% SULPHUR REMOVED					
VISCOSITY cSt	-	-	-	-	
ST MEDIUM	64.4	61.8	65.3	70.3	
IFT	68.4	44.0	59.7	86.5	
ST OIL	68.5	52.5	76.5	69.0	
CMC					
PENTANES 16.24	6.27	6.79	9.53	50.67	
TOLUENES					

SULPHATE MEASUREMENTS

EXPERIMENT # 2

SPECTROPHOTOMETER: Shimadyu

WAVELENGTH: 420 nm

SPECIES:

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
6	1/50	.187	1435.38	23.04
7	1/50	.261	2000.92	32.12
8	1/50	.094	724.62	11.63
9	1/50	.085	655.84	10.53
10	1/50	.100	770.48	12.37

CALCULATIONS:

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 2

SPECIES:

DATE ANALYSED: 870618

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
0 h	34.6	32.5	59.9
24 h	68.5	68.4	64.4
48 h	52.5	44.0	61.8
72 h	76.5	59.7	65.3
96 h	69.0	86.5	70.3

GROWTH EXPERIMENT 2

SPECIES: A. paraffineus

DATE: 870619

INITIAL CONDITIONS:

TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
0	.220	1	1			.39 g/L
24 h	.257	1	2			.39 g/L
48 h	.447	1	3			.5 g/L
72 h	.195	1	4			.1 g/L
96 h	.087	1	5			.2 g/L

PENTANE / TOLUENE INSOLUBLES

EXPERIMENT # 2 -- 870623

SPECIES: Arthrobacter paraffineus

SAMPLE ID	FLASK wet wt	init	diff 1	dry wt	init	diff 2	GOOCH final	init	diff 3	% INSOLUB ((2 + 3)/1)
.1	81.50	80.33	1.17	80.47	80.33	.14	32.220	32.17	.05	16.24
2	83.54	82.54	1.02	82.55	82.52	.103	26.254	26.22	.034	6.27
3	81.03	79.66	1.37	79.40	79.66	.04	21.033	20.98	.053	6.79
4	82.79	81.72	1.07	81.80	81.72	.08	32.732	32.71	.022	9.53
5	82.40	81.35	1.05	81.87	81.35	.52	24.932	24.920	.012	50.67

EXPERIMENT: 3 pH Variations

BEGIN DATE: 870416
END DATE:

FLASK LABELS alpha / numeric 31-40

RANGE:

SPECIES: A. paraffineous

MEDIUM USED: MSM + .01 YE

INITIAL pH: 7.0 / 6.5

TEMPERATURE: 30 degrees/25 degrees

HYDROCARBON SOURCE: Lloydminster Crude

PERSONS INVOLVED: Norah Davidson
Peter Daugulis

COMPUTER INFORMATION:

SULPHATE MEASUREMENTS

EXPERIMENT # 3

SPECTROPHOTOMETER: PYE UNICAM

WAVELENGTH: 420 nm

SPECIES: A. paraffineus

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
32	1	.027	24.383	.3993
37	1	.044	38.042	.6230
38	1	.036	31.408	.514
39	1	.030	26.334	.43
40	1	.022	20.090	.329

CALCULATIONS:

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 3 pH Variations (numeric 31.40)

SPECIES: A. paraffineus

DATE ANALYSED: 870526/870527

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
32	56.1	45.3	82.1
37	52.5	47.4	60.7
38	4.60	37.4	31.0
39	49.2	38.7	73.5
40	39.2	28.4	67.0

EXPERIMENT: 4 BASICS

BEGIN DATE: 870403
END DATE: 870416

FLASK LABELS alpha / numeric

RANGE: A-F

SPECIES: A. paraffineus

MEDIUM USED: MSM+.01

INITIAL pH: 7.0

TEMPERATURE: 25degrees/30 degrees

HYDROCARBON SOURCE: Lloydminster

PERSONS INVOLVED: Norah Davidson
Peter Daugulis

COMPUTER INFORMATION:

SULPHATE MEASUREMENTS

EXPERIMENT #

SPECTROPHOTOMETER: Shimadyu UV160

WAVELENGTH: 420

SPECIES: A. paraffineus

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
A	neat	.032	28.286	
B	"	.166	135.61	
C	"	.079	65.360	
D	"	.073	60.677	
E	"	.037	31.798	
F	"	.063	53.262	

CALCULATIONS:

VISCOSITY MEASUREMENTS

EXPERIMENT #

SPECIES:

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE
A	Too thick	-	-	-
B	Insufficient Sample			
C	400	1.126	629.82	709.18
D	Insufficient Sample			
E	400	1.126	636.11	716.26
F	400	1.126	181.98	204.90

SURFACE AND INTERFACIAL TENSION

EXPERIMENT #

SPECIES:

DATE ANALYSED:

SAMPLE ID		SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
35	A	54.5	40.9	71.7
	B	37.2	16.6	57.0
36	C	37.8	23.5	61.8
	D	37.3	19.0	52.1
34	E	37.5	19.9	54.2
	F	38.1	19.1	49.1

EXPERIMENT: 6 Fermentor no aeration

BEGIN DATE: 870601
END DATE: 870605

FLASK LABELS alpha / numeric

RANGE:

SPECIES: A. paraffineus

MEDIUM USED: MSM + $\frac{SO4}{2}$ + $\frac{YE}{2}$

INITIAL pH: 7.0

TEMPERATURE: 30 degrees C

HYDROCARBON SOURCE: 20% Lloydminster Crude

PERSONS INVOLVED: Norah Davidson
Peter Daugulis

COMPUTER INFORMATION:

FERMENTER

EXPERIMENT # 6 No aeration

SPECIES: A. paraffineus

DATE: 870601

APPARATUS: NBSCo 5l fermentor

INITIAL CONDITIONS: pH 7 + 30C 20% Lloydminster Crude
250 ml inoculum 294 rpm MSM + $\frac{SO4}{2}$ + $\frac{YE}{2}$

PARAMETERS	Initial	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH		6.43	6.27	6.23	6.29	
TEMP		30.25	31.0	28.5	28.0	
OD @620						
DRY WT		.4	.4	.5	.4	
SUBJ.-AQ		+	+++	+++		
SUBJ.-OIL		++	++	++		
VOL AQ HARVESTED		100 ml	100 ml	100 ml	100 ml	
VOL OIL HARVESTED		50 ml	50 ml	50 ml	110 ml	
SO4 ABS @420	1/50 .163	.086	.107	.085	.739	
% SULPHUR REMOVED						
VISCOSITY cSt		612.44	478.55	507.83	467.29	
ST MEDIUM		70.5	55.7	60.9	61.4	61.0
IFT		32.1	25.9	30.3	32.7	29.5
ST OIL		37.9	34.1	37.0	29.7	38.5
CMC						
PENTANES		18.45	15.87	17.90	11.04	62.3
TOLUENES						

GROWTH EXPERIMENT 6

SPECIES: A. paraffineus

DATE: 870619

INITIAL CONDITIONS:

PYE UNICAM

TIME	ABS. @ 620	DIL.	VALUE tin	VALUE final	DRY WT. init	VOL. 10ml	VALUE
0		1	6	1.618	1.618	0	0
24		1	7	1.616	1.612	$\frac{.004}{10ml}$	.4 g/L
48		1	8	1.630	1.625	$\frac{.005}{10ml}$	.5 g/L
72		1	9	1.636	1.631	$\frac{.005}{10ml}$	.5 g/L
96		1	10	1.618	1.614	$\frac{.004}{10ml}$	.4 g/L

VISCOSITY MEASUREMENTS

EXPERIMENT # 6

SPECIES:

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE
(i) 24h	400	1.126	512	612.44
48h	400	1.126	425	478.85
72h	400	1.126	451	507.83
96h	400	1.126	415	467.29

(i) a great deal of water phase noted— separation of 0.1 + sitting period resulted in a 2 layer mixture (0.1 + water)

SULPHATE MEASUREMENTS

EXPERIMENT # 6

SPECTROPHOTOMETER: Shimadyu

WAVELENGTH:

SPECIES:

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
0	50	.163	1251.96	20.10
24	"	.086	663.48	10.65
48	"	.107	823.98	13.23
72	"	.085	655.84	10.53
96	"	.739	5654.04	90.77

CALCULATIONS:

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 6

SPECIES:

DATE ANALYSED: 870619

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
0h	37.9	32.1	70.5
24h	34.1	25.9	55.7
48h	37.0	30.3	60.9
72h	29.7	32.7	61.4
96h	38.5	29.5	61.0

PENTANE / TOLUENE INSOLUBLES

EXPERIMENT # 6

SPECIES: 870623

SAMPLE ID	FLASK wet wt	init	diff 1	dry wt	init	diff 2	GOOCH final	init	diff 3	% INSOLUB ((2 + 3)/1)	
6	82.31	81.28	1.03	81.38	81.28	0.10	40.540	40.45	0.09	.19/1.03	18.45
7	83.74	82.99	0.75	83.05	82.99	0.06	331.79	33.12	0.059	.119/.75	15.87
8	82.93	81.88	1.05	82.01	81.88	0.13	32.229	32.17	0.058	.188/1.05	17.90
9	86.96	86.00	0.96	86.07	86.00	.07	26.258	26.22	.036	.106/.96	11.04
10	83.98	82.98	1.00	83.06	82.98	.58	21.017	20.97	.043	.623/1.00	62.3

FERMENTER

EXPERIMENT # 12 10% crude

SPECIES: A. paraffineus

DATE:

APPARATUS:

INITIAL CONDITIONS:

PARAMETERS	144 DAY 6	168 DAY 7	192 DAY 8	216 DAY9	240 DAY 10
pH	6.70	6.64	6.63	6.48	6.52
TEMP	28 C	28.5 C	28.5 C	28.5 C	29 C
OD @620					
DRY WT	1.0	.6	.8	.7	.5
SUBJ.-AQ	+++	+++	+++	+++	++++
SUBJ.-OIL	+++	+++	+++	+++	++++
VOL AQ HARVESTED	40 ml	40 ml	40 ml	40 ml	40 ml
VOL OIL HARVESTED	-	10 ml	10 ml	10 ml	10 ml
SO4 ABS @420	.038	.034	.052	.044	.073
% SULPHUR REMOVED	.07	.06	.08	.07	.10
VISCOSITY cSt	na	620.6	417.75	530.35	485.30
ST MEDIUM	64.1	65.0	63.5	63.8	63.2
IFT	na	21.2	24.1	25.6	25.8
ST OIL	na	35.5	36.1	36.6	36.2
CMC					
PENTANES	na	16.34	11.96	12.20	10.24
TOLUENES					

GROWTH EXPERIMENT 12

SPECIES: A. paraffineus

DATE: 870629

INITIAL CONDITIONS: .24 filter Sartorius dried in dessicator

	TIME	ABS.	VALUE	INIT.	DRY WT.	VOL.	VALUE
16	24h		1.623	1.615	.008		.8
17	48h		1.658	1.654	.004		.4
18	72h		1.635	1.629	.004		.6
19	96h		1.633	1.625	.008		.8
20	120h		1.637	1.630	.007		.7
21	144h		1.654	1.644	.010		1.0
22	168h		1.662	1.656	.006		.6
23	192h		1.621	1.614	.008		.8
24	216h		1.641	1.634	.007		.7
25	240h		1.623	1.618	.005		.5

SULPHATE MEASUREMENTS

EXPERIMENT # 12

SPECTROPHOTOMETER: Shimadzu

WAVELENGTH: 420

SPECIES: A. paraffineus

SAMPLE ID	DILUTION	SULPHATE READINGS	mg [SO4/L]	% SULPHUR REMOVED
0	neat	0	0	0
24		.003	6.85	.04
48		.038	12.22	.07
72		.051	14.16	.08
96		.039	12.37	.07
120		.035	11.77	.06
144		.038	12.15	.07
168		.034	11.55	.06
192		.052	14.39	.08
216		.044	13.19	.07
240		.073	17.59	.10

CALCULATIONS:

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 12

SPECIES: A. paraffineus

DATE ANALYSED: July 2, 1987

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
24 h	34.6	21.9	64.4
48	35.5	24.0	61.0
72	33.8	22.9	58.9
96	34.9	24.3	58.8
120	34.9	23.0	61.9
144	no sample	no sample	64.1
168	35.5	21.2	65.0
192	36.1	24.1	63.5
216	36.6	25.6	63.8
240	36.2	25.8	63.2

VISCOSITY MEASUREMENTS

EXPERIMENT # 12

SPECIES: A. paraffineus

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE
Day 1 24 h	400	1.126	305	343.43
2	"	"	500	563
3	"	"	123	138.50
4	"	"	445	501.07
5	Stingy - viscometer clogged after			
7	400	1.126	560	630.60
8	"	"	371	417.75
9	"	"	471	530.35
10	"	"	431	485.30

Each sample, when placed in a vial, separated out into approx. 2 ml water phase and approx. 8 ml oil phase.

Crude Oil	400	1.126	375	422.25

PENTANE / TOLUENE INSOLUBLES

EXPERIMENT # 12

SPECIES: A. paraffineus

SAMPLE ID	FLASK wet wt	init	diff 1	dry wt	init	diff 2	GOOCH final	init	diff 3	% INSOLUB ((2 + 3)/1)
1-24 hr	86.41	86.00	.41	86.03	86.00	.03	40.478	40.458	.02	12.2
2-48	79.97	79.59	.38	79.61		.02	40.568	40.540	.028	12.63
3-72	82.30	81.88	.42	81.89		.01	21.013	20.975	.038	11.12
4-96	81.66	81.28	.38	81.30		.02	32.737	32.701	.036	14.74
6-Day5	81.91	81.43	.48	81.45	81.43	.02	74.160	24.922	.038	12.08
7-Day7	83.39	82.98	.41	83.00	82.98	.02	33.172	33.125	.047	16.34
8-Day8	80.89	80.33	.56	80.34	80.33	.01	21.037	20.980	.057	11.96
9-Day9	82.50	82.00	.50	82.02	82.00	.02	32.746	32.708	.041	12.20
10-Day10	80.76	80.34	.42	80.35	80.34	.01	90.490	40.457	.033	10.24

EXPERIMENT: 13 20% Oil, 10% inoculum - Nutrient Broth

BEGIN DATE: 87.07.07
END DATE:

FLASK LABELS - alpha / numeric

RANGE:

SPECIES: A paraffineus

MEDIUM USED: msm + 1.0 g/L L/E 42

INITIAL pH: 7.0

TEMPERATURE: 30°

HYDROCARBON SOURCE: 1L (20%) Lloydminster Crude

PERSONS INVOLVED: Norah Davidson, Peter Daugulis

COMPUTER INFORMATION:

FERMENTER

EXPERIMENT # 13

SPECIES: A paraffineus

DATE:

APPARATUS: NBS

INITIAL CONDITIONS: pH + temp. 30°

PARAMETERS	initial probe	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH		6.45/6.63	7.10/6.43	6.94/6.69	/6.98	/6.98
TEMP		29.0	29.0	29.5	28.15	28.5
OD @620		.678	.795	1.13	1/2 .388	1/2 .311
DRY WT		.3	.6	1.0	.6	.5
SUBJ.-AQ		+++	+++	+++	+++	+++
SUBJ.-OIL		+	++	++	++	++
VOL AQ HARVESTED		25ml	25ml	25ml	25ml	25ml
VOL OIL HARVESTED		13ml	13ml	13ml	13ml	13ml
SO4 ABS @420		.544	.281	.285	.299	.367
% SULPHUR REMOVED		.277	.147	.147	.155	.1974
VISCOSITY cSt	403.12	392.97	493.19	541.61	585.52	647.45
ST MEDIUM		67.12	64.0	64.9	63.1	66.8
IFT						
ST OIL		22.1	19.9	22.8	24.8	32.8
CMC		33.5	30.3	32.7	32.4	37.8
PENTANES		17.02	9.56	14.35	21.45	32.54
TOLUENES						

FERMENTER

EXPERIMENT # 13

SPECIES: A. paraffineus

DATE: 87-07-07

APPARATUS: Stirred tank

INITIAL CONDITIONS:

PARAMETERS	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
pH	6.88	6.84	6.89	6.87/7.33	6.90
TEMP	20°C	29	29.5	29	29
OD @620	½ .247	½ .308	½ .282	½ .270	.887 neat
DRY WT	.3	.5	.5	.3	.3
SUBJ.-AQ	+++	+++	++	++	
SUBJ.-OIL	+++	++	++	++	
VOL AQ HARVESTED	25ml	25ml	25ml	25ml	25ml
VOL OIL HARVESTED	13ml	13ml	13ml	13ml	13ml
SO4 ABS @420	.548	.342	.446	.366	.458
% SULPHUR REMOVED	.297	.179	.239	.193	.246
VISCOSITY cSt	629.43	748.79	789.37	892.92	537.10
ST MEDIUM	64.6	64.6	65.3	65.0	66.0
IFT	31.3	21.7	23.7	24.1	23.1
ST OIL	33.9	36.2	33.6	36.7	33.3
CMC					
PENTANES	12.41	10.20	29.26	13.86	
TOLUENES					

GROWTH EXPERIMENT
EXPERIMENT # 13

SPECIES: A. paraffineous

DATE: 87-07-08

INITIAL CONDITIONS: filter 0.2 sartarius

TIME	620 ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
24hr	/678	neat	1.611-1.605	0.003	10ml	.3g/L
48hr	.795	neat		.006	10ml	.6
72hr	1.13	neat	1.610-1.600	.01	10ml	1g?1
96hr	0.388	1:1	1.603-1.597	.006	10ml	.6
120hr	.310	1:1	1.656-1.651	.005	10ml	.5
144hr	.247	1:1	1.624-1.621	.003	10ml	.3
168hr	.308	1:1	1.622-1.617	.005		.5
192hr	.282	1:1	1.632-1.627			.5
216hr	.270	1:1	1.631-1.628			.3
240hr	.587	neat	1.621-1.618			.3

DATE: 87-07-08

SULPHATE MEASUREMENTS

EXPERIMENT # 13

SPECTROPHOTOMETER:

WAVELENGTH: 420 mm

SPECIES: A. paraffineus

SAMPLE ID	pH	DILUTION	SULPHATE READINGS	mg [SO4/L]	% SULPHUR REMOVED
1		0	.547/.546/.545/.544	76.8	.277
2		0	.292/.296/.281	40.0	.144
3	6.94	0	.292/.290/.293/.285	40.6	.147
4	7.23	0	.304/.307/.310/.299	42.9	.155
5	7.5	0	.387/.390/.392/.367	53.6	.194
6		0½	.285/.284/.274/.548	82.3	.297
7	7.22	½	.171/.171/.172/.171/.342	49.7	.179
8	7.21	½	.238/.239/.223/.446	66.1	.239
9	7.33	½	.210/.219/.183/.366	53.5	.193
blank		½	.230/.235/.237/.229/.458	68.0	.246
10			.007/.008		

CALCULATIONS:

C = 158.42 x Abs x Dil - 4.5089

$$\% S = \frac{[\ ] \times 4.0}{\frac{3}{346.00 \times 10}} \times 100 = \frac{[\ ] \times 01.25}{346}$$

(#1 C=(152.79 x A x dil-blank) - 6.3278

PENTANE / TOLUENE INSOLUBLES

DATE: 87-07-08

EXPERIMENT # 13 - -

SPECIES: A. paraffineus

	SAMPLE ID	FLASK wet wt	init	diff 1	dry wt	init	diff 2	GOOCH final	init	diff 3	% INSOLUB ((2 + 3)/1)
1	24hrs	86.54	86.00	.54	86.13	86.0	.13	32.247[1]	32.202	.045	
2	48hrs	81.03	80.34	.69	80.32	80.34	0 4	32.782	32.716	.066	9.56
5	24rs	80.23	79.66	.57	79.67	79.66	.01 3	26.388	26.301	.087	17.02
	72hr	82.40	81.78	.62	81.80	81.78	.02 5	25.041	24.972	.069	14.35
	96hr	82.59	81.35	1.24	81.54	81.35	.19 2	33.192	33.116	.076	21.45
	120hr	84.22	82.53	1.69	83.07	82,53	.54 7	32.234	32.233	.01	32.54
	144hr	03.53	82.99	.54	83.02	82.99	.03 4	32.759	32.722	.037	12.41
	168hr	80.15	79.66	.49	79.67	79.66	.01 5	33.177	33.136	.041	10.20
	192hr	83.66	81.35	2.31	82.01	81.35	.066	32.224	32.208	0.16	29.26
	216hr	82.22	81.78	.44	81.80	81.78	.02 5	32.740	32.699	.041	13.86
	240hr	84.22	82.52	1.70	82.80	82.52	2	25.01	24.958		

DATE: 87-07-07

VISCOSITY MEASUREMENTS

EXPERIMENT # 13

SPECIES: A. paraffineus

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE
Crude	400	1.126	358	403.12
24hr	400	1.126	349	372.97
48hr	400	1.126	432	493.19
72hr	400	1.126	481	541.61
96hr	400	1.126	520	585.52
120hr	400	1.126	575	647.45
144hr	400	1.126	559	629.43
168hr	400	1.126	665	748.79
192hr	400	1.126	701	789.33
216hr	400	1.126	793	892.92
240	400	1.126	477	537.10

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 13

SPECIES:

DATE ANALYSED:

SAMPLE ID	pH	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
1	6.45	33.5	22.1	67.2
2	7.10	30.3	19.9	64.0
3	6.94	32.7	24.8	63.1
4	7.23	32.4	24.8	63.1
5	7.5	37.8	32.8	66.8
6	7.25	33.9	31.3	64.6
7	7.20	36.2	21.7	64.6
8	7.21	33.6	23.7	65.3
9	7.33	36.7	24.1	65.0
10	7.30	33.3	23.1	66.0

EXPERIMENT: #14

BEGIN DATE: 87-07-07
END DATE:

FLASK LABELS alpha / numeric

RANGE: a_1-n_8

SPECIES: Desulfovibrio desulfuricans

MEDIUM USED: AP1/AP. no sulphate

INITIAL pH:

TEMPERATURE: 25°

HYDROCARBON SOURCE: Lloydminster Crude/DBT in paraffin 5g/L
DBT in Dodecane 20g/L

PERSONS INVOLVED: Norah Davidson
Peter Daugulis

Tube with 8ml medium, 1 ml inoculum, 1ml hydrocrabon, stirred once very 24 hr. Inoculum grown on thioglycollate broth

COMPUTER INFORMATION:

DATE: 87-07-07

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye Unicam

WAVELENGTH: 440

SPECIES: D. desulfuricans

	SAMPLE ID	pH	DILUTION	ABSORBANCE	% SULPHUR REMOVED
Apl	w/oal medium	6.70	0	.044	
	a2 dodecane	6.55	0	.163	1.24
	a3 paraffin	5.76	0	.183	6.05
	a4 crude	5.82	0	.192	.16
Apl	" a5	6.78	10	.076	
	a6	6.40	010	.080	.16
	a7	6.38	10	.087	4.46
	a8	6.41	10	.080	.02
	100 g/ml		neat	.075	
	200 g/ml		neat	.198	
	0blank		neat	.015	

CALCULATIONS:

DATE: 87-07-09

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye Unicam

WAVELENGTH: 440

SPECIES: D. desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
Std 0	0	.008	
Std 30	0	.236	
Std 60	0	.377	
6_1	0	.036	
6_2	0	.134	1.47
6_3	0	.132	3.69
6_4	0	.185	.15
6_5	10	.087	
6_6	10	.100	1.39
6_7	10	.136	25.26
6_8	10	.121	.43

CALCULATIONS:

DATE: 87-07-10

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye unicam

WAVELENGTH: 440

SPECIES: Desulfuricoms

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
0 mg/L	neat	.011	
C_1	neat	.033	
C_2	neat	.135	1.00
C_3	neat	.202	7.57
C_4	neat	.163	.15
C_5	10	.099	
C_6	10	.102	.02
C_7	10	.105	1.73
C_8	10	.117	.21

CALCULATIONS:

DATE: 87-07-12

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye

WAVELENGTH:

SPECIES:

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	-	.025	
d_1	0	.043	
d_2	0	.182	1.57
d_3	0	.160	4.84
d_4	0	.186	.16
d_5	10	.092	
d_6	10	.108	1.70
d_7	10	.082	
d_8	10	.069	0

CALCULATIONS:

DATE: 87-07-13

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye

WAVELENGTH: 440

SPECIES: D d

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
control	0	.010	
e_1	0	.085	
e_2	0	.268	2.12
e_3	0	.181	3.7
e_4	0	.149	.05
e_5	10	.099	
e_6	10	.153	7.00
e_7	10	.120	.08
e_8	10	.108	.08

CALCULATIONS:

DATE: 87-07-13

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye Unicam

WAVELENGTH: 440

SPECIES: D desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	0	.001	
f_1	0	.065	
f_2	0	.226	1.82
f_3	0	.263	9.3
f_4	0	.128	.05
f_5	10	.088	
f_6	10	.180	12.21
f_7	10	.104	7.19
f_8	10	.108	.24

CALCULATIONS:

DATE: 87-07-14

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER Pye Unicam

WAVELENGTH: 440

SPECIES: D desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	0	.016	
g_1 cont1	0	.054	
g_2	0	.250	2.29
g_3	0	.301	11.96
g_4	0	.203	.17
g_5 cont1	10	.067	
g_6	10	.096	3.58
g_7	10	.090	11.03
g_8	10	.089	.26

CALCULATIONS:

DATE: 87-07-15

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye unicam

WAVELENGTH: 420

SPECIES: D. desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank		0.27	
h_1	cont1	.102	
h_2		.248	1.61
h_3		.491	19.74
h_4		.213	.11
h_5	cont1	.071	
h_6		.133	8.10
h_7		.141	36.77
h_8		.113	.54

CALCULATIONS:

DATE: 87-07-16

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye Unicam

WAVELENGTH: 420

SPECIES: Desulfovibrio

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	neat	.001	
i_1	"	.037	
i_2	"	.216	2.06
i_3	"	.363	16.28
i_4	½	.128	.26
i_5	10	.088	
i_6	"	.110	2.62
i_7	"	.116	13.76
i_8	"	.094	.04

CALCULATIONS:

GROWTH EXPERIMENT

EXPERIMENT #14

SPECIES: Desulfurobrio desulphuricans

DATE: 87-07-08

INITIAL CONDITIONS: Pye Unicam

TIME	620m, ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
24hr						
a_1	.003					
a_2	.023	.020				
a_3	.027	.024				
a_4	.027	.024				
a_5	.077					
a_6	.084	.004				
a_7	.111	.034				
a_8	.128	.051				

GROWTH EXPERIMENT

EXPERIMENT #14

SPECIES: Dd

DATE: 87-07-13

INITIAL CONDITIONS: Pye Unicam @ 620
dispo Falcon tubes

TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
blank	.024					
e_1	.026					
e_2	.058	.032				
e_3	.053	.027				
e_4	.-93	.067				
e_5	.106					
e_6	.181	.075				
e_7	.157	.050				
e_8	.155	.049				

DATE: 87-07-13

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye Unicam

WAVELENGTH: 440

SPECIES: D desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	0	.001	
f_1	0	.065	
f_2	0	.226	1.82
f_3	0	.263	9.3
f_4	0	.128	.05
f_5	10	.088	
f_6	10	.180	12.21
f_7	10	.104	7.19
f_8	10	.108	.24

CALCULATIONS:

DATE: 87-07-14

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER Pye Unicam

WAVELENGTH: 440

SPECIES: D desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	0	.016	
g_1 cont1	0	.054	
g_2	0	.250	2.29
g_3	0	.301	11.96
g_4	0	.203	.17
g_5 cont1	10	.067	
g_6	10	.096	3.58
g_7	10	.090	11.03
g_8	10	.089	.26

CALCULATIONS:

DATE: 87-07-15

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye unicam

WAVELENGTH: 420

SPECIES: D. desulfuricans

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank		0.27	
h_1 cont1		.102	
h_2		.248	1.61
h_3		.491	19.74
h_4		.213	.11
h_5 cont1		.071	
h_6		.133	8.10
h_7		.141	36.77
h_8		.113	.54

CALCULATIONS:

DATE: 87-07-16

SULPHIDE MEASUREMENTS

EXPERIMENT # 14

SPECTROPHOTOMETER: Pye Unicam

WAVELENGTH: 420

SPECIES: Desulfovibrio

SAMPLE ID	DILUTION	ABSORBANCE	% SULPHUR REMOVED
blank	neat	.001	
i_1	"	.037	
i_2	"	.216	2.06
i_3	"	.363	16.28
i_4	½	.128	.26
i_5	10	.088	
i_6	"	.110	2.62
i_7	"	.116	13.76
i_8	"	.094	.04

CALCULATIONS:

GROWTH EXPERIMENT

EXPERIMENT #14

SPECIES: Desulfurobrio desulphuricans

DATE: 87-07-08

INITIAL CONDITIONS: Pye Unicam

TIME	620m, ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
24hr						
a_1	.003					
a_2	.023	.020				
a_3	.027	.024				
a_4	.027	.024				
a_5	.077					
a_6	.084	.004				
a_7	.111	.034				
a_8	.128	.051				

GROWTH EXPERIMENT

EXPERIMENT #14

SPECIES: Dd

DATE: 87-07-13

INITIAL CONDITIONS: Pye Unicam @ 620
dispo Falcon tubes

TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
blank	.024					
e_1	.026					
e_2	.058	.032				
e_3	.053	.027				
e_4	.-93	.067				
e_5	.106					
e_6	.181	.075				
e_7	.157	.050				
e_8	.155	.049				

GROWTH EXPERIMENT
EXPERIMENT # 14

SPECIES: Dd

DATE: 87-07-14

INITIAL CONDITIONS:

TIME	620 ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
f_1 control	.009					
f_2	.037	.028				
f_3	.062	.53				
f_4	.056	.047				
f_5 control	.093					
f_6	.222	.129				
f_7	.075	0				
f_8	.184	.091				

GROWTH EXPERIMENT
EXPERIMENT #14

SPECIES: D. desulforibrio

DATE: 87-07-16

INITIAL CONDITIONS: Pye Unicam @ 620 through dispo. tubes

TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
blank	0					
i_1 API withput	.006					
i_2	.051	.045				
i_3	.064	.058				
i_4	.374	.368				
i_5	.116					
i_6	.150	.034				
i_7	.168	.052				
i_8	.228	.112				

EXPERIMENT: #15 Airlift

BEGIN DATE: 87-07-15
END DATE: 87-07-18

FLASK LABELS alpha / numeric

RANGE: EXP 15 24-96hr

SPECIES: Arthrobacter paraffineus

MEDIUM USED: 1.0g?1 22.51

INITIAL pH: 7.00

TEMPERATURE: Room temp. approx. 22°C -25

HYDROCARBON SOURCE Lloydminster Crude 2.5 l (10%)

PERSONS INVOLVED: Norah Davidson,
Peter Daugulis

COMPUTER INFORMATION:

FERMENTER

EXPERIMENT # 15

SPECIES: Ap

DATE: 87-07-15

APPARATUS: Airlift

INITIAL CONDITIONS:

PARAMETERS	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH	7.03	7.15	7.20		
TEMP	25	25	24		
OD @620	.493	.909	.913		
DRY WT	.4	.8	1.2		
SUBJ.-AQ	+	+	+		
SUBJ.-OIL	½+	½+	½+		
VOL AQ HARVESTED	50ml	30ml	30ml		
VOL OIL HARVESTED	50ml	20ml	20ml		
SO4 ABS @420	.139	.122	.094		
% SULPHUR REMOVED	.169	.143	.112		
VISCOSITY cSt	na	na	1101.23		
ST MEDIUM	60.7	60.9	60.1		
IFT	23.9	38.6	39.5		
ST OIL	35.7	36.4	37.5		
CMC					
PENTANES	11.86	11.81	94.67		
TOLUENES					

GROWTH EXPERIMENT

EXPERIMENT # 15 AIRLIFT

SPECIES: Ap

DATE:

INITIAL CONDITIONS:

TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
24hr	.493	neat	1.619	.004	10ml	.4 g/L
48hr	.909	neat	1.661	.008	10ml	.8 g/L
72hr	.713	neat	1.652	.012	10ml	1.2 g/L

SULPHATE MEASUREMENTS

EXPERIMENT # 15 AIRLIFT

SPECTROPHOTOMETER:

WAVELENGTH:

SPECIES:

SAMPLE ID	pH	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
24hr	7.03	½	.083		
48hr	7.15	½	.065		
72hr	7.20	½	.055		
	24hr		.027 blank .166-.027 = .129	17.51	.169
	48hr		.130-.008 = .122	14.82	.143
	72hr		.110-.008 = .102	11.65	.112

CALCULATIONS:

PENTANE / TOLUENE INSOLUBLES

EXPERIMENT # 15··

SPECIES: A. paraffineus

SAMPLE ID	FLASK wet wt	init	diff 1	dry wt	init	diff 2	GOOCH final	init	diff 3	% INSOLUB ((2 + 3)/1)
24h	87.18	86.00	1.18	86.03	86.00	.03	32.240	32.130	.110	$\frac{.14}{1.18}$ x 100 =11.8
48h	83.82	82.99	.83	83.03	82.99	.04	32.789	32.731	.058	11.81
72h	80.56	79.66	.90	79.69	79.66	.03	33.169	32.237	.822	94.67

VISCOSITY MEASUREMENTS

EXPERIMENT #

SPECIES:

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE
24h	400	1.126	Too watery (water phase not separable)	+
48h	400	1.126	"	+
72h	400	1.126	978	1101.23

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 15

SPECIES:

DATE ANALYSED:

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
24hr	35.7	23.9	60.7
48hr	36.4	38.6	60.9
72h	37.5	39.5	61.1

EXPERIMENT: 16 Stirred Tank N2

BEGIN DATE: 870720
END DATE:

FLASK LABELS alpha / numeric

RANGE:

SPECIES: Desulfibrio desulfuricans

MEDIUM USED: MSM TYE 4L

INITIAL pH: 7.0

TEMPERATURE: 29

HYDROCARBON SOURCE: Lloydminster Crude 1L

PERSONS INVOLVED:

295 rpm

1.0 litre/min, i.e. 0.2 v/v nitrogen

24 hr control

then inoculated with Ad

COMPUTER INFORMATION:

FERMENTER

EXPERIMENT # 16 Stirred Tank - N2

SPECIES: Desulfovibrio after 24 hr

DATE:

APPARATUS:

INITIAL CONDITIONS:

PARAMETERS	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH	6.58	6.83			
TEMP	29	29.5			
OD @620	.226	.220			
DRY WT g/L	.3	.4			
SUBJ.-AQ					
SUBJ.-OIL					
VOL AQ HARVESTED					
VOL OIL HARVESTED					
SO4 ABS @420	0	.050			
% SULPHUR REMOVED	0	.013			
VISCOSITY cSt	1062.94	na			
ST MEDIUM	83.4	67.3			
IFT	30.6	47.8			
ST OIL	35.9	52.1			
CMC					
PENTANES	na	na			
TOLUENES					

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 16

SPECIES:

DATE ANALYSED:

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
24h	35.9	30.6	
48h	52.1	47.8	

VISCOSITY MEASUREMENTS

EXPERIMENT # 16

SPECIES:

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE
24 hr	400	1.126	944	1062.94
48 hr	400	1.126	-	too thick

FERMENTER

EXPERIMENT # 17 Airlift - N2

SPECIES: none - blank

DATE: 870722

APPARATUS: 9L/min

INITIAL CONDITIONS: 15L MSM 1.9/L pH 7.0
1.5L oil
11L/min N2

PARAMETERS	DAY 1	OUT OF N2 DAY 2	DAY 3	DAY 4	DAY 5
pH	6.58	7.31			
TEMP	25°C	25°C			
OD @620	.180	.423			
DRY WT	.2 g/L	.01 g/L			
SUBJ.-AQ					
SUBJ.-OIL					
VOL AQ HARVESTED	20 ml	20 ml			
VOL OIL HARVESTED	20 ml	20 ml			
SO4 ABS @420	.030	.059			
% SULPHUR REMOVED	.0025	.0513			
VISCOSITY cSt	410.99	811.65			
ST MEDIUM	64.5	67.1			
IFT	22.9	33.3			
ST OIL	35.0	36.4			
CMC					
PENTANES					
TOLUENES					

SULPHATE MEASUREMENTS

EXPERIMENT # 17

SPECTROPHOTOMETER:

WAVELENGTH:

SPECIES:

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
A		.030	0.124	.0025
B		.059	4.84	.0513

CALCULATIONS:

EXPERIMENT: 18 6.5L Stirred Tank Fermentor

BEGIN DATE: September 14
END DATE: September 19

FLASK LABELS alpha / numeric

RANGE: A-I

SPECIES: Arthrobacter paraffineus

MEDIUM USED: MSM + SO_4 + 0.25 YE

INITIAL pH: 7.32

TEMPERATURE: 30^0

HYDROCARBON SOURCE: paraffin 10BT

PERSONS INVOLVED: Suzanne Rioux
George Ching

COMPUTER INFORMATION:

GROWTH EXPERIMENT 18

SPECIES:

DATE:

INITIAL CONDITIONS:

	TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
A		0.126	-				
B		0.082	-				
C		0.155	-				
D		0.087	10				
E		0.175	10				
F		0.063	10				
G		0.106	10				
H		0.116	10				
I		0.067	10				

SULPHATE MEASUREMENTS

EXPERIMENT # 18

SPECTROPHOTOMETER: PYE UNICAM

WAVELENGTH: 420

SPECIES:

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
A	100	0.155	3400	
B	100	0.105	2450	
C	100	0.091	2200	
D	100	0.149	3300	
E	100	0.224	4840	
F	100	0.183	4050	
G	100	0.151	1460	
I	100	0.265	2610	

CALCULATIONS:

SULPHATE MEASUREMENTS

EXPERIMENT # 18

SPECTROPHOTOMETER: PY UNICAM

WAVELENGTH: 440

SPECIES: Arthrobacter paraffineus

SAMPLE ID	DILUTION	SULPHATE READINGS	[SO4/L]	% SULPHUR REMOVED
A	10	0.243	12.3	
B	10	0.075	4.6	
C	10	0.220	10.8	
D	10	0.111	6.0	
E	10	0.252	129	
F	10	0.118	65	
G	10	0.140	75	
H	10	0.165	85	
I	10	0.149	79	

CALCULATIONS:

VISCOSITY MEASUREMENTS

EXPERIMENT # 18

SPECIES:

SAMPLE ID	VISCOMETER	C	TIME (sec)	VALUE	
A	400	1.126/40°	82	92.3	
			76	85.6	
			74	83.3	
			71	79.9	x = 84.66
			73	82.2	
B	400	1.126/40°	79	89.0	
			77	86.7	
			75	84.5	
			75	84.5	x = 85.84
			75	84.5	
C	400	1.126/40°	88	99.1	
			81	91.2	
			78	87.8	x = 90.3
			78	87.8	
			76	85.6	
D	400	1.126/40°	70.4	79.3	
E	400	1.126/40°	67.9	76.5	
F	400	1.126/40°	71.4	80.4	
G	400	1.126/40°	78.7	88.6	
H	400	1.126/40°	77.2	86.9	
I	400	1.126/40°	79.6	89.6	
J	40°	1.126/40°	82.1	92.5	

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 18

SPECIES:

DATE ANALYSED:

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
A	34.7	28.9	58.1
B	31.5	33.1	67.2
C	31.9	17.0	41.8
D	32.4	7.1	61.2
E	30.2	23.9	69.7
F	33.6	18.1	63.4
G	33.4	8.9	61.1

FERMENTER

EXPERIMENT #

SPECIES:

DATE:

APPARATUS:

INITIAL CONDITIONS:

PARAMETERS	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
	A	B	C	D	E	F	G	H	I	J
pH	7.00	7.08	6.96	7.09	6.66	6.65	6.62	5.87	5.19	5.13
TEMP	31	27	30	30	29.5	31	31	31		
OD @620	.239	.090	.251	.198	.191	.449	.358	.260	.275	.701
DRY WT										
SUBJ.-AQ										
SUBJ.-OIL										
VOL AQ HARVESTED										
VOL OIL HARVESTED										
SO4 ABS @420	.196	.125	.251	.213	.250	.198	.200	.256	.304	.285
% SULPHUR REMOVED										
VISCOSITY cSt										
ST MEDIUM	62.1	62.1	62.2	63.0	61.6	66.0	69.4	54.9	70.8	64.0
IFT	36.2	34.0	16.0	9.4	34.1	7.4	7.9	13.8	20.3	20.7
ST OIL	27.2	27.2	33.6	26.6	30.4	31.0	34.3	31.6	31.4	30.5
CMC										
PENTANES										
TOLUENES										

EXPERIMENT: 2

BEGIN DATE: September 29/87
END DATE: October 3/87

FLASK LABELS alpha / numeric

RANGE: A-L

SPECIES: Arthrobacter paraffineus

MEDIUM USED: MSM +

INITIAL pH: 7.00

TEMPERATURE: 31°

HYDROCARBON SOURCE: DBT/paraffin

PERSONS INVOLVED: Suzanne Rioux
George Ching

COMPUTER INFORMATION:

GROWTH EXPERIMENT

SPECIES: Arthrobacter paraffineus

DATE:

INITIAL CONDITIONS:

ID	TIME	ABS.	DIL.	VALUE	DRY WT.	VOL.	VALUE
A	0	0.239					
B	4	0.090					
C	14	0.251					
D	20	0.198					
E	24	0.191					
F	40	0.449					
G	48	0.368					
H	64	0.260					
I	72	0.275					
J	88	0.701					
K	96	0.635					
L	112	0.545					

SULPHATE MEASUREMENTS

EXPERIMENT # 2

SPECTROPHOTOMETER:

WAVELENGTH: 440

SPECIES: Arthrobacter paraffineus

SAMPLE ID	DILUTION	SULFIDE READINGS		[$S^{=}$/L]	% SULPHUR REMOVED
		Blank	+Reagent		
A		0.448	0.432	-	
B		0.161	0.135	-	
C		0.160	0.157	-	
D		0	0.418	4.5	
E		0.327	0.317	-	
F		0.765	0.760	-	
G		0.285	0.281	-	
H		0.373	0.379	-	
I		0.189	0.202	0.7	
J		0.776	0.746	-	
K		1.003	0.999	-	
L		0.906	0.882	-	

CALCULATIONS:

SULPHATE MEASUREMENTS

EXPERIMENT # 2

SPECTROPHOTOMETER:

WAVELENGTH: 420

SPECIES: Arthrobacter paraffineus

SAMPLE ID	DILUTION	SULPHATE READINGS	x10' [SO4/L]	% SULPHUR REMOVED
A	100	0.196	21.4	
B	100	0.125	14.4	
C	100	0.251	26.9	
D	100	0.213	18.3	
E	100	0.250	21.5	
F	100	0.198	17.1	
G	100	0.200	17.3	
H	100	0.156	13.4	
I	100	0.304	26.7	
J	100	0.285	24.3	
K	100	0.249	21.5	
L	100	0.382	33.0	

CALCULATIONS:

SURFACE AND INTERFACIAL TENSION

EXPERIMENT # 2

SPECIES: Arthrobacter paraffineus

DATE ANALYSED: September 29/87

SAMPLE ID	SURFACE TENSION OIL dynes/cm	INTERFACIAL TENSION dynes/cm	SURFACE TENSION MEDIUM dynes/cm
A	27.2	36.2	62.1
B	27.2	34.0	62.1
C	33.6	16.0	62.2
D	26.6	0.4	63.0
E	30.4	34.1	61.6
F	31.0	7.4	66.0
G	34.3	7.9	69.4
H	31.6	13.8	54.9
I	31.4	20.3	70.8
J	30.5	20.7	64.0
K	32.2	15.5	63.7
L	32.8	29.0	70.0